Effect of *Sm1* on End-use Quality of Durum Wheat (*Triticum turgidum* L. *var durum*)

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By

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ABSTRACT

Genetic resistance to the orange wheat blossom midge (*Sitodiplosis mosellana;* OWBM*)* is an important breeding target to prevent yield and quality losses of durum wheat produced in western Canada. To date, only a single characterized midge resistance gene, *Sm1,* has been identified. *Sm1* confers antibiosis resistance to the OWBM. It has been genetically localized to chromosome 2BS of hexaploid wheat (*Triticum aestivum* L.). *Sm1* has been introgressed into locally adapted germplasm. Currently, no *Sm1* carrying durum wheat lines are available for commercial production, and no studies have characterized the influence of *Sm1* on yield and end-use quality of durum wheat. The main objectives of this study were: 1) To determine the effect of *Sm1* on grain yield and end-use quality. 2) To genetically map the *Sm1* introgression. For this work, 122 $F_{5:9}$ recombinant inbred lines (RILs) derived from a cross between the midge susceptible durum wheat cultivar CDC Verona (*Sm1 "-"*) and resistant experimental line DT780 (*Sm1 "+"*). Agronomic and end-use quality traits of the mapping population were analyzed. The results from each environment were used for quantitative trait loci (QTL) analysis at Kernen (SK) in 2009 and 2010, and at Indian Head (SK) in 2009. On average, the presence of *Sm1* was associated with higher grain yield and yellow pigment content, but lower kernel weight, reduced grain protein content, and weaker gluten properties. However, it was possible to identify RIL lines carrying *Sm1* that expressed higher kernel weight, grain protein content, and stronger gluten. A genetic linkage map spanning 58 cM on chromosome 2B near *Sm1* was constructed. QTL mapping suggested that the total length of the *Sm1* introgression into durum wheat was approximately 11cM. Nearly all traits measured showed QTLs associated with *Sm1*. For grain protein content, a QTL proximal to *Sm1* was identified, suggesting that *Sm1* per se may not be contributing to the reduced grain protein observed in the *Sm1* carriers of the RIL mapping population. The results presented here suggest that on average, *Sm1* is associated with higher grain yield

and some reduced end-use quality factors, but that it may be possible to combine *Sm1* with high grain yield and end-use quality equivalent to current check cultivars.

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LIST OF ABBREVIATIONS

AACC:	American Association of Cereal Chemists
AAFC:	Agriculture and Agri-food Canada
ANOVA:	Analysis of variance
BU:	Brabender Units
CIM:	Composite Interval Mapping
CIMMYT:	International Maize and Wheat Improvement Center
CTAB:	hexadecyltrimethyl-ammonium bromide
CWAD:	Canadian Western Amber Durum
EST:	Expressed Sequence Tag
FN:	Falling Number
GI:	Gluten Index
Gli:	Gliadin
Glu:	Glutenin
GPC:	Grain Protein Content
HD:	Heading date
HMW:	High Molecular Weight
LMW:	Low Molecular Weight
HMW-GS:	High Molecular Weight Glutenin Subunits
LMW-GS:	Low Molecular Weight Glutenin Subunits
HRSW:	Hard Red Spring Wheat
LOD:	logarithm of odds ratio
LSM:	Least Square Mean
MQM:	Multiple QTL Mapping
OWBM:	Orange Wheat Blossom Midge
PCR:	Polymerase chain reaction
PH:	Plant Height
QTL:	Quantitative Trait Loci

RLX:	Relaxation
SSR:	Simple sequence repeats
SSR:	Single Strand
ST:	Stretching Time
SSCP:	Single-stranded Conformation Polymorphism
TKW:	Thousand-kernel Weight
TWT:	Test Weight
WETG:	Wet Gluten Content
YLD:	Yield
YP:	Yellow Pigment
α-gliadin:	alpha gliadin
β-gliadin:	beta gliadin
γ-gliadin:	gamma gliadin
ω-gliadin:	omega gliadin

1.0 Introduction

The majority of Canadian durum wheat (*T. turgidum* L. *var. durum*) is produced in Saskatchewan. Durum wheat is primarily used for pasta production (Distelfeld et al. 2006). The orange wheat blossom midge (OWBM; *Sitodiplosis mosellana* Ge´hin) is a major pest in Canadian wheat, and is present in most global wheat growing areas (Lamb et al. 2000b). The OWBM larvae feed on developing wheat kernels, and cause economic loss through lower grain yield and reduced end-use quality (Lamb et al. 2000b). OWBM damaged kernels are characterized by high grain protein content, dark flour color, high flour ash, and weak gluten properties. Efforts to introgress *Sm1* into durum wheat are a priority, because the OWBM is becoming increasingly prevalent in parts of the Western Canada durum growing regions.

Genetic resistance of wheat midge damage is desirable compared to chemical control (Elliott, 1988; Oakley et al. 1998), and effective host resistance has been identified (Barker and McKenzie, 1996) in two forms: antixenosis and antibiosis, for deterrence of oviposition and inhibition of larval growth, respectively. The most characterized resistance is associated with a single, partially dominant resistance gene designated as *Sm1* (McKenzie et al. 2002), and is associated with antibiosis properties (Thomas et al. 2005). *Sm1* temporarily induces an increased level of natural phenolic compounds in the seed coat to deter midge feeding (Ding et al. 2000), and this is believed to be the physiological basis for resistance (Thomas et al. 2005). Once the larvae have been killed, the defensive chemical in the seed drops to the same levels as in susceptible seeds (Lamb, 2003). Several hexaploid wheat cultivars have been released in Canada that carry *Sm1* and their popularity is increasing because the insect pest is becoming more prevalent in all Western Canada wheat growing regions.

Currently, no midge-tolerant durum wheat (*Triticum turgidum* L.) cultivars are grown in Canada, despite the introgression of *Sm1* into adapted durum wheat genetic backgrounds. One reason for the slowed acceptance is that Western Canadian durum

wheat breeding programs have noted anecdotal evidence, suggesting an association of *Sm1* with reduced end-use quality in early attempts to develop midge tolerant cultivars. However, there is little published information available on the effects of *Sm1* on economically important quality traits in durum wheat. Thus, it is not clear if this association with reduced quality is due to linkage drag or pleiotropic effects. This study was designed to assess the effect of *Sm1* on the end-use quality performance, and to determine the genetic association of *Sm1* with end-use quality of durum wheat.

2.0 Literature Review

2.1 Durum Wheat

Durum wheat is an allotetraploid (2n = 4x = 28) with seven homoeologous chromosome pairs (AABB) (Nachit et al. 2001). The A genome in durum wheat originated from the diploid wild wheat (einkorn) (*Triticum urartu* Thum. Ex Gandil.); the B genome is believed to have been derived from *Aegilops speltoides* (Tausch) (Gooding and Davies, 1997). Durum wheat is milled to produce semolina and is used predominantly for pasta production (Olmos et al. 2003; Blanco et al. 2006). Some durum wheat production is used for baking, but because of the absence of the D genome found in hexaploid wheat (*T. aestivum* L.; AABBDD), baking potential is reduced (Kerber and Tipples, 1969). Canadian durum wheat is classified into four Canada Western Amber Durum (CWAD) wheat milling grades using standards set by the Canadian Grain Commission (Dexter and Edwards, 1998). Durum wheat possesses unique quality characteristics that differentiate it from other classes of wheat. Durum semolina is valued for its bright yellow pigment that results from elevated levels of lutein (Ramachandran et al. 2010), making it an important end-use quality trait of durum (Pozniak et al. 2007). In general, good potential pasta quality is also related to high protein content and strong gluten properties (Clarke et al. 2009). Sissons (2004) indicated that test weight, thousand-kernel weight, weather damage (sprouting), vitreousness, and visual appearance of the grain are important end-use quality traits.

Historically, durum wheat has been grown in Mediterranean climates of North Africa, southern Europe, Turkey, and Syria because durum production and end-use quality is better suited to semi-arid climates. Durum wheat was introduced into western Canada in the late 19th Century (Dexter, 2008). In Canada, durum wheat production is in the drier, southern regions of the prairies of Manitoba, Saskatchewan, and Alberta. In Canada, two sub-classes of durum wheat are recognized: conventional cultivars with moderate gluten strength, and extra-strong cultivars with strong gluten

properties similar to the USA desert (Clarke et al. 2005). Conventional strength durum makes pasta with good cooking quality, whereas stronger gluten cultivars can enhance cooking tolerance and have good blending capability. Currently, extra-strong gluten durum cultivars are marketed in an identity preserved system for quality assurance purposes (AAFC, 2009).

2.2 Orange Wheat Blossom Midge

2.2.1 Overview: OWBM and Wheat Production

The orange wheat blossom midge (*Sitodiplosis mosellana;* OWBM) can be a major pest of wheat *(Triticum* spp.). It has been found in most of the wheat-growing areas of the world (Barnes, 1956). The OWBM can cause significant yield loss and reduced end-use quality of wheat (Lamb et al. 2000b). However, it is difficult to estimate the risk of damage because infestations vary from year to year due to climatic conditions. Infestations caused by wheat midge were initially identified in the East-Central area of Saskatchewan and expanded to most of the Parkland area of Saskatchewan, with severe impacts on spring wheat, durum wheat, spring rye and triticale production in these areas since 1983 (Elliott and Mann, 1996; Elliott et al. 2011).

Midge larvae feed on developing wheat kernels, causing shriveling, cracking, and deformation (Lamb et al. 2000a; Elliott et al. 2011). The damage is not readily apparent, however, external changes manifest in kernel color, size and shape. Midge feeding may result in abortion of some kernels, while other damaged kernels do not fully develop. Extremely damaged kernels are small and light, and may pass through the combine with the chaff during harvest. This and aborted kernels cause the majority of the yield loss associated with OWBM. In addition, midge damaged kernels are likely to remain in harvested samples, which can reduce the grade of the harvested wheat. OWBM damaged wheat is characterized by kernels with high protein content, dark flour color, high flour ash, weak sticky dough properties and reduction in flour yield (Dexter and Edwards, 1997). Surface discoloration of kernels

is of particular concern for durum wheat, because aesthetic appearance is very important for commercial pasta products (Dexter and Edwards, 1998). The Canadian Grain Commission has established tolerance levels for midge damage for Canada Western Amber Durum: 2% limit for No. 1 CWAD and 8 % limit for No. 2 CWAD (Canadian Grain Commission; www.grainscanada.gc.ca).

2.2.2 The Life Cycle of OWBM

The life cycle of the OWBM is presented in Figure 1. The female midge is a tiny fly with an orange abdomen. Adult midge emerges from the pupal stage in the soil over a 5-6 week period. In western Canada, emergence occurs from mid-June to mid-July when wheat spikes are emerging from the sheath and beginning to flower. Environmental conditions play an important role in wheat midge activity and dispersal. Warm, calm, humid weather is ideal for midge flight and ovipositing. The adult midge is not a strong flier, and its flight is regulated by light intensity for vertical migration. Rainfall may also limit flight activity (Pivnick and Labbe´, 1992). Wheat midge has six stages of oviposition on wheat spikes: arriving, probing, inserting the ovipositor, walking, sitting, and departing (Ganehiarachchi and Harris, 2007; 2009). During the day, the OWBM remains within the crop canopy where conditions are humid. Midge oviposition takes place from an hour before to a half hour after sunset, provided the temperature is above 14–15° C and wind speeds are less than 10 km per hour (Pivnick and Labbe´, 1993). Cloudy conditions may allow an earlier onset of oviposition (Pivnick and Labbe´, 1992). Moreover, wheat at a susceptible stage (Zadoks growth stages 52-60: Zadoks et al. 1974), namely, the period of spikelet initiation is attractive to OWBM for oviposition (Reeher, 1945; Barnes, 1956; Doane et al. 1987; Basedow, 1973; Elliott and Mann, 1996; Ding and Lamb, 1999). Midge females readily lay eggs throughout this period, but oviposition drops substantially by 10 days after anthesis (Lamb et al. 1999). Egg-laying requires air temperatures above 10–11°C (Barnes, 1956; Mukerji, et al. 1988; Smith and Lamb, 2001). There is a positive association between the number of eggs laid per spike, and the length of time the female midge

spends on a spike (Ganehiarachchi and Harris, 2009). The short susceptible stage of wheat usually coincides with the short flight period and oviposition of the female midge, as well as infestation by larvae (Lamb et al. 1999). Lamb et al. (1999) also found that larvae exhibited selectivity to feed on developmental stage of wheat kernels. Some spikes escape infestation due to offset of the midge flight and heading time (Wright and Doane, 1987; Kurppa, 1989; Lamb et al. 1999). Therefore, differences in heading date of wheat may reduce oviposition and infestation by wheat midge.

After oviposition, females rarely migrate down from the spikes to the lower level they occupied during the day (Pivnick and Labbe, 1992). Eggs are laid either between glumes or within florets, and on spikelet surfaces just prior to, or at anthesis (Lamb et al. 2003). The eggs incubate for four to seven days until hatching, and then larvae move from the spikelet surface into the florets to feed on developing grain for approximately 2-3 weeks (Ding and Lamb, 1999). The developing kernels are damaged by the feeding of the first two larval instars, while the third instar adults do not feed (Pivnik and Labbe, 1993). The mature larvae remain in the wheat spike enclosed in a transparent skin. The larvae then drop to the soil surface, burrow into the soil, and form overwintering cocoons. Overwintered larvae begin to pupate in Spring near the soil surface, and the adult flies emerge approximately two weeks later, completing the life cycle.

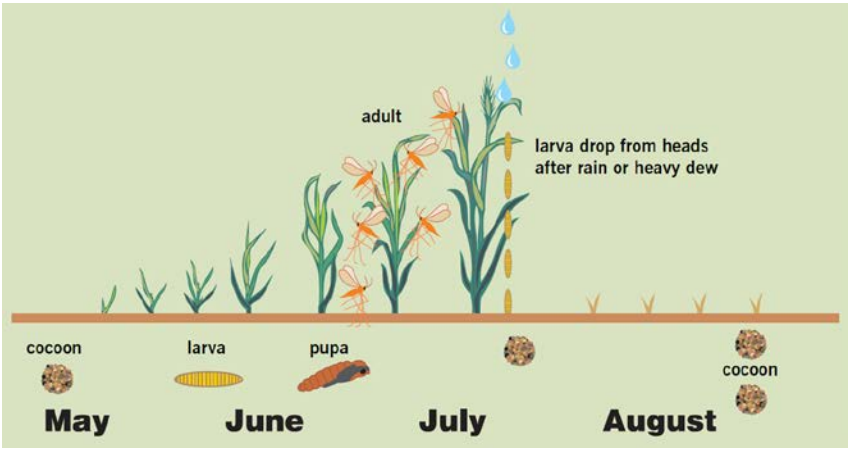

Figure 1: Life cycle of the orange wheat blossom midge (Extension Entomology, NDSU)

2.2.3 Wheat Midge Genetic Control and Management

2.2.3.1 Agronomic Management

In Canada, total losses due to the OWBM have been estimated to exceed $100 million under high midge pressure (Thomas et al. 2005). Wheat yield can be reduced by approximately 15 percent by midge infestation of 4 to 5 per spike (Government of Saskatchewan, http://www.agriculture.gov.sk.ca). Control of the wheat midge has traditionally been achieved through appropriate agronomic practices, and the use of insecticides. Continuous wheat cropping should not be practiced, because this accelerates the build-up of midge populations and other pests. Seeding date has been proven an effective tool to minimize damage due to the OWBM, particularly for early maturing wheat cultivars (Government of Saskatchewan, http://www.agriculture.gov.sk.ca). In general, early seeding can minimize midge damage because plants pass through the susceptible stage before the adult wheat midge emerges from the soil (Government of Saskatchewan, http://www.agriculture.gov.sk.ca). Insecticides are effective at controlling the OWBM, and are recommended when there is at least one adult midge for every four or five wheat spikes (Lamb et al. 2000a). Several insecticides are available for control of the OWBM, including those containing chloropyrifos (Lorsban®, Nufos ®, and Pyrinex ®), which control adult flies as well as eggs (Government of Saskatchewan, http://www.agriculture.gov.sk.ca). However, uniform insecticide coverage of spikes is essential for the control of eggs and larvae. Evening application is recommended although early morning applications may also provide acceptable control. A disadvantage of insecticidal control of the OWBM is that the active ingredients will also severely reduce populations of *Macroglenes penetrans* (Kirby) (Hymenoptera: Pteromalidae), a parasitoid wasp (Gharalari, 2008). This small parasitic wasp is known to keep populations of OWBM in check (Gharalari, 2008). Eggs of the parasitic wasp remain dormant inside the wheat midge larva over the winter, and then destroy the larva the following spring (Gharalari, 2008).

2.2.3.2 Host Resistance

Host resistance is a desirable option for controlling OWBM (McKenzie et al. 2002). The use of genetic resistance to the OWBM is preferred, because chemical control is expensive (Elliott, 1988), and application timing is critical for effective control (Oakley et al. 1998). The inheritance of OWBM resistance in durum wheat has been investigated, which could be incorporated through breeding. Genetic resistance to OWBM has been identified for both antixenosis and antibiosis, which inhibit oviposition and larval growth, respectively (Thomas et al. 2005). The complete resistance mechanism is likely controlled by more than one gene, for antixenosis and antibiosis properties, with complementary interaction among genes (Gharalari et al. 2009a). Hence, incorporation into breeding programs will be a challenge.

However, other factors may also be involved in conditioning resistance. Escaping OWBM attack may be due to differences in flowering time, regulated by other genes besides the mechanism of antixenosis and antibiosis resistance. Two important components of flowering time are the vernalization (*Vrn*) and photoperiod (*Ppd*) genes, which control the growth and developmental phases of wheat (Herndl et al. 2008).

2.2.3.3 Antixenosis for Oviposition Deterrence of OWBM

Oviposition deterrence (antixenosis) to female wheat midge has been confirmed in durum (Lamb et al. 2001) and common wheat (Lamb et al. 2002; Ganehiarachchi and Harris 2007; Fox et al. 2009), and promises to be useful for reducing wheat midge damage. The presence of antixenosis generally influences where oviposition occurs on the wheat spike. In terms of oviposition deterrence, the female midge probes less frequently and inserts their ovipositors into spikelets much less frequently, and spends less time when compared to susceptible hosts, thus reducing the impact of oviposition (Ganehiarachchi and Harris, 2009). The physiological mechanism of antixenosis is not well understood, but oviposition deterrence could be due to the wheat plant lacking stimuli for female midge to oviposit. Female midge

also shows oviposition preference through chemical or morphological characteristics of the wheat spike (Gharalari et al. 2009b).

Lamb et al. (2000a) reported that some wheat lines deter oviposition due to less preference by female midge. Oviposition deterrence caused at least a 50% reduction in egg density (Lamb et al. 2000a; Lamb et al. 2002). Meanwhile, Lamb et al. (1999, 2000b) also reported that a 70% reduction in oviposition could lower larval infestation levels to below the economic threshold required for insecticidal control. It is difficult to assess oviposition deterrence, because egg density is difficult to measure precisely and variability of oviposition by females is not easily manipulated (Smith and Lamb, 2001).

Gharalari et al. (2009b) suggested that the production of wax on plant surfaces might be associated with antixenosis because lines demonstrating antixenosis were consistently waxier at the post-anthesis stage than susceptible lines. Similarly, Cervantes et al. (2002) found that wax production and chemical composition influenced oviposition deterrence of Hessian fly. However, there is no evidence showing the effect of wax on oviposition deterrence of wheat midge, and no promising trait correlated to oviposition deterrence could be considered for wheat breeding programs (Gharalari et al. 2009b). The expression of antixenosis is controlled by multiple genetic factors and complementary gene interactions (Amri et al. 1990; Pani and Sahu, 2000; Gharalari et al. 2009b). The mechanism of antixenosis is still not fully understood, but may also involve the expression of antibiosis genes (Gharalari et al. 2010). However, there is no evidence for linkage between antixenosis genes and the antibiosis gene, *Sm1* (Gharalari et al. 2009b), although some lines were observed to have both the antixenoisis and antibiosis traits (Lamb et al. 2000a). It would be challenging to incorporate antixenosis genes in wheat breeding programs because of the multigenic nature of the trait and environmental effects (Gharalari et al. 2009b). However, commercial wheat cultivars that express the antixenosis gene either alone or pyramided with the antibiosis gene may provide an increased level of resistance to OWBM (Gharalari et al, 2009b).

2.2.3.4 Antibiosis Resistance

Genetic resistance to the OWBM has been studied for over a decade, but only a single partially dominant gene has been identified. A single resistance (antibiosis) gene, designated as *Sm1*, located on chromosome 2BS, was first identified in the hexaploid winter wheat cultivars "Caldwell", "Howell", "Augusta", and "Seneca" (Barker and McKenzie, 1996). Female midge still infests resistant cultivars by ovipositing on the surface of exposed spikes. Hatched larvae begin to feed on developing seeds, but are later deterred by antibiotic compounds in the seed immediately after anthesis (Ding et al. 2000; Ding and Lamb, 1999; Elliott and Mann, 1996). The resistant lines decrease larval density 59-100%, and reduce seed damage by 70-100% compared to susceptible lines in spring wheat (Lamb et al. 2000a).

At a physiological level, *Sm1* appears to confer resistance by elevating levels of phenolic acids (p-coumaric and ferulic) in the bran of grains induced by feeding larvae. Resistant lines produce both compounds at higher concentrations than their susceptible counterparts (Abdel-Aal et al. 2001). Abdel-Aal et al. (2001) found that ferulic acid, the major phenolic acid in wheat grains, was significantly correlated with resistance to OWBM in hexaploid wheat. The level of phenolic acid in uninfected seeds between resistant and susceptible lines may differ just after anthesis (Karban and Baldwin, 1997). During the first five days after anthesis, resistant lines under midge attack produce consistently higher concentrations of ferulic acid than susceptible lines (Ding et al. 2000). The mechanism that triggers the production of phenolic acids does not operate if midge larvae are not feeding on the seed. Fortunately, the increased levels of phenolic compounds are unlikely to influence end-use quality as levels of phenolic acid return to normal as the seeds mature (Ding et al. 2000; Lamb et al. 2000b).

Several studies have also confirmed significant correlations between phenolic acid content in grains and their resistance to insects and diseases (Arnason, 1992; Cabrera, 1995; McKeehen et al. 1999). Phenolic acids cross-linked with carbohydrates in the cell walls in cereals are believed to contribute a physical barrier against invasive insects and microorganisms (Abdel-Aal et al. 2001). Phenolic acids

are commonly found in many cereal grains. In previous studies, high contents of phenolic acids were observed to accumulate in the outer layers of the bran in wheat kernels (Baublis, et al. 2000; Baublis, et al. 2002; Onyeneho and Hettiarachchy, 1992). Phenolic acids can be classified into two derivatives of either hydroxycinnamic acid or hydroxybenzoic acid (Figure 2). Significantly higher antioxidative efficiency is contributed from the CH=CH-COOH group in the hydroxycinnamic acids rather than the -COOH group in the hydroxybenzoic acids (White and Xing, 1997). The most abundant phenolics acids in wheat kernels are ferulic, vanillic, p-coumaric, and syringic acids (Sosulski et al. 1982). Ferulic acid (4-hydroxy-3-methoxycinnamic acid) exists primarily in the outer layers of cereal grains and constitutes up to 90% of the total phenolic acids in wheat (Sosulski et al. 1982). Among the various phenolic acids, p-coumaric acid, ferulic acid and other hydroxycinnamic acids have been found to have better antioxidant activities than hydroxybenzoic acids (Andreasen et al. 2000; Emmons et al. 1999; Baublis, et al. 2000; Onyeneho and Hettiarachchy, 1992; Yu et al. 2003).

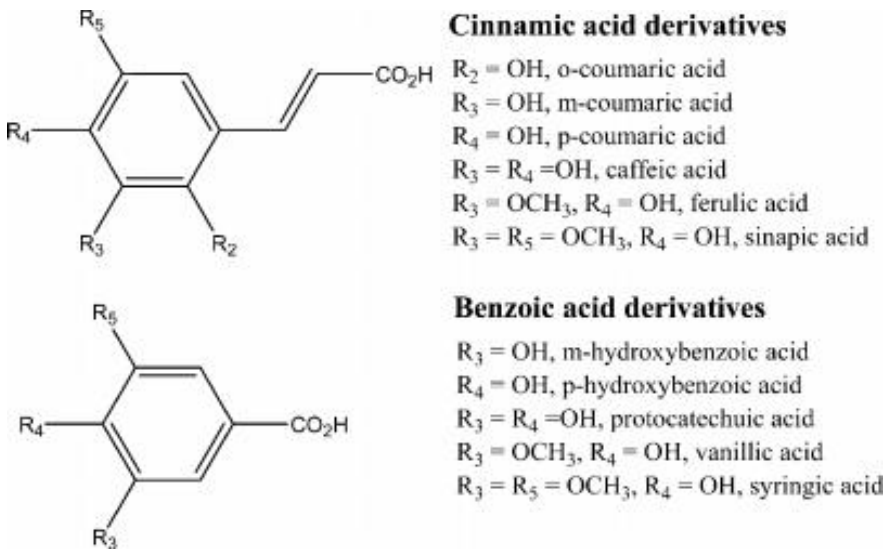

Figure 2: Chemical structures of monomeric phenolic acids (Mattila et al. 2005; Andreasen et al. 2000)

2.2.4 Midge Resistant Cultivars and Deployment of A Genetic Interspersed Refuge System

Sm1 was transferred from winter into hexaploid spring wheat (McKenzie et al. 2002) and commercialized in hexaploid wheat cultivars in western Canada. The *Sm1* resistance gene is a very valuable resource for wheat production. As noted above, *Sm1* temporarily induces elevated levels of phenolic compounds to resist midge feeding, consequently reducing the survival of OWBM (Ding et al. 2000). 'Goodeve', one of the first Canadian Western Red Spring (CWRS) wheat cultivars released carrying *Sm1,* was selected by application of the DNA marker WM1 (fragment size was 232 base pairs; forward: CAC CTG GAA TGT TGG ACT G and reverse: ACA TCA TCT GTC AAC GCA CTA) (Thomas et al. 2005). 'Glencross' was the first DH cultivar in the Canada Western Extra Strong (CWES) wheat class with *Sm1*, and was selected using molecular marker-assisted selection with the WM1 marker on haploid plants prior to chromosome doubling (DePauw et al. 2011). Currently, *Sm1* has also been transferred to durum wheat (Clarke et al. 2010), but midge resistant durum wheat genotypes have not yet been commercialized due to the strict end-use quality requirements for the Canada Western Amber Durum (CWAD) wheat class.

There is a concern that the resistance might become ineffective over time because populations of OWBM virulent on *Sm1*-expressing wheat genotypes are known to exist. Theoretically, the evolution of virulent biotypes of pests can be delayed significantly by pyramiding several resistance genes compared to the use of single sources of resistance (Gould, 1986). However, no other antibiosis genes have been discovered. The resistance of *Sm1* may be prolonged through the use of refuges (McKenzie et al. 2002; Smith et al. 2004). As such, current hexaploid wheat cultivars released for production to producers contain an interspersed refuge of a susceptible variety (10%) in varietal blends to delay the evolution of resistance to the *Sm1* by reducing the frequency of homozygous *Sm1*-virulent midge in the population (Gould, 1986; Rausher, 2001). Without an interspersed refuge system, midge

tolerance based on *Sm1* could break down within 10 years (Midge Tolerant Wheat; http://www.midgetolerantwheat.ca). The interspersed refuge system helps prevent the build-up of virulent midge populations and could extend the life of midge tolerance from 10 years to 90 years or longer (Midge Tolerant Wheat; http://www.midgetolerantwheat.ca).

The current cultivars and their susceptible blends are presented in Table 1. Similarly, this strategy could be used in the development of durum wheat cultivars containing *Sm1*. However, this refuge system does not guarantee that 10 percent of the refuge cultivar will suffer severe a heavy midge infestation. In order to maintain effective midge resistance, seed producers and farmers are both responsible for preserving midge tolerance by signing a Midge Tolerant Wheat Stewardship Agreement that limits the use of farm-saved seed to one generation past Certified Seed (Midge Tolerant Wheat; http://www.midgetolerantwheat.ca). Combination of antixenosis and antibiosis genetic resistance could also be a mechanism to inhibit evolution of virulent wheat midge (Gould, 1986). Thus, oviposition deterrence (antixenosis) would be a desirable trait to combine with antibiosis (Lamb et al. 2000b).

Table 1: Available midge tolerant wheat cultivars, currently used as a "varietal blend" (Midge Tolerant Wheat; http://www.midgetolerantwheat.ca) (VB stands for varietal blend).

"Varietal Blend" (Interspersed Refuge System)		
Class[a]	**90% Tolerant Cultivars**	**10% Susceptible Cultivars**
CWRS	AC® Unity VB and AC® Fieldstar VB	AC® Waskada
CWRS	AC® Goodeve	AC Intrepid
CWES	AC® Glencross	AC® Burnside
CWRS	AC® Shaw VB	AC Domain
CWRS	CDC Utmost VB	Harvest
CPSR	AC® Enchant VB	AC Crystal
CWRS	AC® Vesper VB	AC® Waskada
CPSR	AC® Conquer VB	5701PR

[a] **CWRS:** Canada Western Red Spring Wheat; **CWES:** Canada Western Extra Strong Wheat; **CPSR:** Canada Prairie Spring Wheat

2.3 Physical Damage on Grain Quality

2.3.1 Effect of Pre-harvest Sprouting Damage

There are four sources of formation of α-amylase activity during grain development which can lead to high activity in harvested grain: pre-harvest sprouting which is the breakdown of grain dormancy as a result of late harvest in wet conditions (Flintham and Gale, 1988); pre-maturity sprouting of high moisture kernels during early grain development (Flintham and Gale, 1988; Lunn et al. 2001a; Lunn et al. 2001b); α-amylase accumulation in the endosperm cavity (Flintham and Gale, 1988; Evers et al. 1995), and pericarp alpha-amylase activity of wheat grain due to unripe grains from late-developing tillers (Kettlewell and. Cashman, 1997; Olered, 1976; Lunn et al. 2001a; 2001b). Pre-harvest sprouting can also be considered as post-dormancy germination, which usually occurs when harvest is delayed by wet weather beyond the time of dormancy break (Belderok, 1968). Meanwhile, pre-dormancy germination is rare and may be associated with insect damage (Oakley, 1993; Lunn et al. 1995).

According to the Official Grain Grading Guide (Canadian Grain Commission; http://www.grainscanada.gc.ca), broken rootlets within (or extending beyond) the contour of the germ are considered as sprouted kernels. Pre-harvest sprouting is one of the major abiotic constraints influencing the production of high quality grain. Pre-harvest sprouting results in reduction of functional grain quality and economic value by impacting test weight, vitreousness, soundness and processing quality (McCaig et al. 2006). Pre-harvest sprouting is also accompanied by an increase of a-amylase activity, with a reduction in processing grain quality due to partial starch digestion. Higher α-amylase activity is detectable in flour milled from sprouted wheat grain when compared to non-sprouted grain (Singh, 2008). The flour from sprouted wheat results in sticky dough (Mares et al. 2004), and higher levels of discoloration in noodles (Hatcher and Symons, 2000) compared to sound flour. Neither pasta firmness

nor pasta stickiness is affected by sprout damage (Dexter et al. 1990; Donnelly, 1980; Dick et al. 1974).

2.3.2 Effect of Midge Damage

Midge damaged kernels, as defined for grading purposes, must exhibit at least two of following features: a rupture of the bran on either the back or side of the kernel, and/or a distinct white line or mark on the back or side of the kernel (Canadian Grain Commission; http://www.grainscanada.gc.ca). Many damaged kernels are lost at harvest due to seed shriveling, but a large proportion remaining does contribute to quality loss (Miller and Halton, 1961). Thus, midge infestation is a serious cause of sprouting, resulting in yield and economic losses (Lamb et al. 2000b). Several studies have shown that OWBM can severely reduce the end-use quality of wheat samples (Kurppa, 1989; Lamb et al. 2002). Midge damage occurs when the OWBM larvae feed on developing wheat kernels by exuding alpha-amylase to release sugars from the grain (Oakley et al. 1998). Helenius and Kurppa (1989) found that the Hagberg Falling Number, a measure of sprouting tolerance, was negatively correlated with the proportion of damaged grains, and noted that midge damage resembled sprouting damage. Lunn et al. (1995) also concluded that sprouting of grain results from the interaction between midge-damaged kernels and weather conditions. Sprouting occurs in mature kernels when subjected to proper moisture, temperature and timing. Midge damaged kernels exhibit increased water uptake and sprouting under poor weather conditions, leading to reduced end-use quality due to weak and sticky dough properties (Oakley, 1994; Fenney et al. 1988). However, midge damaged kernels have a higher grain protein content (Feillet and Dexter, 1996; Dexter and Edwards, 1998).

Generally, midge damage has a serious effect on wheat dough handling properties and semolina milling performance. In hexaploid wheat, severe midge damaged kernels have a high protein content, poor milling quality and there is a loss of dough functionality due to the presence of insect proteases and increased wheat α-amylase activity (Dexter and Edwards, 1998). As midge damage increased, the ash content and

speck count increased, while color declined and pasta became less bright and undesirably brown (Dexter and Edwards, 1997; 1998). However, gluten quality, measured by the SDS-sedimentation test and pasta texture did not appear to be affected by midge damage (Dexter and Edwards, 1998).

2.4 Durum Wheat Grain Quality

End-use quality characteristics of durum wheat differ between products, but generally include minimum levels of protein, high milling yield, yellow flour color and moderately strong gluten (El Ouafi et al. 2001). Durum wheat quality consists of specific physical and chemical quality attributes, and rheological and processing characteristics. Physical grain quality traits consist of components, such as test weight, kernel weight, and proportion of vitreous kernels (Clarke et al. 1998). Chemical grain quality traits include falling number, yellow pigment content, protein content, and gluten quality.

2.4.1 Physical Grain Quality

2.4.1.1 Test Weight

Test weight is common measure of soundness of wheat and has been used as an indicator of overall grain quality. Sound wheat refers to plump, fully mature kernels, free of damaged kernels, and with high test weight. However, there is no consensus on the utilization of test weight as an indicator of wheat milling yield, even though a positive association was found between lower test weights and reduced semolina milling yield (Dexter and Edwards, 1999). Test weight also can be influenced by kernel shape (Campbell et al. 1999).

2.4.1.2 Thousand-kernel Weight

Thousand-kernel weight is a measure of average kernel size (Giura and Saulescu, 1996). Large kernels can produce more vigorous seedling, and may contribute to

higher grain yield (Botwright et al. 2002; Blair, 1992; Chastain et al. 1995). Thousand-kernel weight can be affected by the kernel shape, kernel length, kernel width, and other factors (Campbell et al. 1999; Dholakia et al. 2003). Test weight and kernel weight of durum wheat have high heritabilities, and these two traits can be selected in early generations, based on stable genotypic differences over environments (Clarke et al. 2009).

2.4.2. Chemical Grain Quality

2.4.2.1 Yellow Pigment Content

Yellow pigments are responsible for the color of semolina, which is an important end-use quality trait in international markets (Troccoli et al. 2000). Semolina color of durum wheat is an important quality criterion with regards to pasta production. Yellow amber color is preferred, due to increased global competition in pasta market, so color has become more important trait in durum wheat breeding programs (Dexter and Marchylo, 2001). Yellow pigment content of durum wheat endosperm is comprised primarily of carotenoids (lutein, zeaxanthin and β-cryptoxanthin) (Cenci et al. 2004, Panfili et al. 2004, El Ouafi et al. 2001). Carotenoid pigments contribute the bright yellow color of durum wheat semolina, mainly comprising lutein in a non-esterified form (Hentschel et al. 2002). Ramachandran (2010) indicated carotenoids are the only known class of compounds contributing considerably to yellow pigment. Furthermore, high positive correlation coefficients (0.94-0.99) of total carotenoids with total yellow pigment have been reported (Digesu et al. 2009, Panfili et al. 2004; Abdel-Aal et al. 2007; Fratianni et al. 2005; Digesu et al. 2009). In addition, Ramachandran (2010) reported a correlation of 0.99 between lutein and yellow pigment content. Ramachandran (2010) also found that lutein expressed as a proportion of total yellow pigment were different among groups, 54% of the total yellow pigment in high and intermediate pigment groups and 38.3% of the total yellow pigment in low pigment groups. In addition, a higher zeaxanthin to lutein ratio was correlated to lower yellow pigment was confirmed (Ramachandran, 2010; Abdel-Aal et al., 2007, Fratianni et al.

2005; Okarter et al. 2010). Similar results were also reported for hexaploid wheat (Hidalgo and Brandolini, 2008; Moore et al. 2005; Roose et al. 2009; Okarter et al. 2010).

Carotenoids are antioxidant compounds that help to reduce the oxidative damage to biological membranes by scavenging peroxyradicals (Troccoli et al. 2000). Carotenoids also play an important role in human health. Lutein, zeaxanthin and β-cryptoxanthin account for almost 85% of total carotenoids in wheat and are related to prevention of age-related macular degeneration of the eye and cardiovascular disease (Mares-Perlman et al. 2002). Lutein and zeaxanthin may also increase dietary iron absorption from wheat and corn based food (Garcial-Casal, 2006).

Higher pigment content is an important breeding objective in durum wheat. Clarke et al. (2006) reported that semolina yellow color was highly heritable and quantitatively inherited. Reported heritability values ranged from 0.90 to 0.97 (Nachit et al. 1995), 0.48 to 0.99 for 16 environments (El Ouafi et al. 2001), 0.34 to 0.95 (Clarke et al. 2006), and 0.97 to 0.99 (Reimer et al. 2008). Meanwhile, the degree of yellowness is also modified by the growing conditions and the milling extraction rate (Borrelli et al. 1999).

The complex inheritance of pigment presents challenges for conventional breeding, so identification of DNA markers linked to the major factors could facilitate parental and progeny selection (Clarke et al. 2006). QTLs for yellow pigment content in durum wheat were identified on Chromosome 7BL linked to marker *Xgwm* 344 (El Ouafi et al. 2001), 2A and 2B (Joppa and Williams, 1988b), 1A, 3B, 5B, 7A and 7B (Patil et al. 2007), 7BL (Zhang and Dubcovsky, 2008) and 7A (Singh et al. 2009). Additional QTLs have been detected on chromosomes 5A (Hessler et al. 2002), 1B and 6A (Zhang et al. 2005), and chromosomes 2A, 4B, and 6B (Pozniak et al. 2007). Four candidate genes coding for a key enzyme (phytoene synthase) in carotenoids synthesis were identified on group 5 (*Psy2*) and group 7 (*Psy1*) chromosomes (Pozniak et al. 2007). *Psy1-A1* identified by Singh et al. (2009) was associated with phenotypic variation in grain yellow pigment in all environments in two of three mapping populations. *Psy1-A1* has also been reported

in many bread wheat cultivars (Zhang and Dubcovsky, 2008). Reimer et al. (2008) showed that the SSR marker *Xgwm146* was strongly linked to *Psy1-B1* confirming that group 7 chromosomes played an important role in yellow pigment expression in durum wheat based on association mapping.

2.4.2.2 Falling Number

The Hagberg Falling Number method is based upon the rapid gelatinization of a suspension of flour in a boiling water bath (95 °C), which measures liquefaction of starch by the starch degrading enzyme alpha-amylase (Hagberg, 1960). It can be used as measure for the endosperm quality at harvest time, classification and quality control, by determining α-amylase activity in grain (Hagemann and Ciha, 1984). Falling number is directly related to the soundness of wheat. Falling number values fluctuate widely depending on the degree of ripening and the amount of rainfall prior to harvest (Mares, 1993). There are actually no standards for falling number, because it is not an official grading factor in the U.S. Federal Grain Inspection Service (FGIS) grain inspection and grading process (Sorenson, 2006). Higher levels of α-amylase are present in sprouted wheat (Kruger, 1994), resulting in a lower falling number. However, some wheat genotypes may have high sprout damage with high falling number indicative of low α-amylase activity (Humphreys and Noll, 2002). Generally, falling number values above 350 seconds are indicative of low enzyme activity and sound wheat grain (Sorenson, 2006). In contrast, falling number values below 200 seconds indicate high levels of enzyme activity (Sorenson, 2006). Hence, minimum falling number values are often included in specifications for grain and flour shipments in commercial trade. Minimum falling number values of 300 to 350 seconds have been requested in contracts for commercial trade (AACC, 2000; Sorenson, 2006). However, waxy wheat has extremely low falling numbers (Abdel-Aal etal. 2002; Graybosch et al. 2000), a phenomenon that is caused by the presence of low-amylose or amylose-free endosperm starch (Graybosch et al. 2000). Falling number estimates wheat starch viscosity by measuring the partial starch

degradation, which manifests as a decrease in starch viscosity when heated to 95°C. Waxy starches swell readily and become viscous initially, but the pastes disintegrate rapidly due to the lack of amylose (Abdel-Aal et al. 2002; Graybosch et al. 2000). The falling number of waxy wheat is extremely low because of the starch pasting properties, despite the presence of α-amylase levels that are similar to sound wheat (Graybosch et al. 2000). Consequently, waxy wheat should be separated from the wheat commercial market because of its low falling numbers. This indicates that other methods should be used, since the Falling Number test is not reliable to test waxy wheat.

2.4.2.3 Grain Protein Content

Grain protein content is one of the most important factors determining pasta and bread-making quality, and is also important to human nutrition (Olmos et al. 2003). High grain protein is valued in export markets desiring good end-use quality (Dohlman and Hoffman, 2000). Thus, high grain protein content is a primary target for hard common wheat and durum wheat breeding-programs. Wheat protein composition is extremely complex with different properties and functions in the grain. In general, good cooking quality is related to a high level of protein and gluten content or an intermediate content of protein with high gluten quality (D'Egidio et al. 1990). An association was found between increased grain protein content and an increase in the amount of gluten proteins (Dexter and Dronzek, 1975). However, this relationship is not consistent (Ciaffi et al. 1991).

Grain protein content of durum wheat is recognized as an important characteristic for pasta cooking quality (D'Egidio et al. 1990; Ciaffi et al. 1991; Blanco et al. 1996; El Ouafi, 2001). Wheat protein content higher than 13% was reported to produce a satisfactory final product, whereas protein content lower than 11% gave a very poor product (Matveef, 1966; Clarke, 2001). Cooked pasta produced from semolina with high protein content has strong physical properties, such as elasticity, extensibility and resistance to overcooking. Similarly, the opposite occurs in cooked pasta made from

low protein grain (Dexter and Edwards, 1998). As grain protein content decreases, pasta become more sticky (Marchylo et al. 1998). The physicochemical characteristics of high grain protein content can be modified by high temperature drying of pasta (De Stefanis and Sgrulletta, 1990). Thus, high temperature drying technology is required and used to improve the cooking quality, color and nutritional quality of pasta (Mercier and Hyberg, 1995; De Stefanis and Sgrulletta, 1990).

Grain protein content expression can be strongly influenced by environmental effects, such as soil fertility, rainfall or temperature (Blanco et al. 2006; Daniel and Triboi, 2000; Suprayogi et al. 2009). As well, grain protein content can be influenced by other physiological factors, such as, nitrogen uptake, assimilation, and remobilization from leaves and stems to the grain during grain filling (People and Dalling, 1988; Feller and Fischer, 1994; Suprayogi et al, 2009). Grain protein content can be increased by nitrogen application, and the development of cultivars with genetically superior grain protein content.

Grain protein content is negatively correlated with grain yield. Selection for high protein usually results in low yield (Cox et al. 1985; Steiger et al. 1996). However, the Canadian durum wheat cultivar, 'Strongfield', consistently displays high grain protein content coupled with high yield in Canadian environments (Clarke et al. 2005). Dexter and Matsuo (1977) found that an increase in grain protein content was associated with an increase in pigment color in two durum cultivars. Furthermore, QTL studies have been used in an attempt to dissect the different loci governing grain protein content and to provide selection tools for breeders (Blanco et al. 1996; Prasad et al. 1999). In durum, QTLs for grain protein content were reported on chromosomes 2B, 5B, 6B, and 7A (Suprayogi et al. 2009), 4BS, 5AL, 6AS, 6BS, and 7BS (Blanco et al. 1996), and 1B, 2BS, 3BL, 4AL, 5AS, 5BL, and 7AS (Conti et al. 2011). For instance, the *Gpc-B1* allele for high grain protein content was identified in tetraploid wild emmer wheat (*Triticum turgidum ssp. Dicoccoides*) (Olmos et al. 2003). Subsequent QTL studies showed that *Gpc-B1* provided an average protein increase of 14 g kg-1 and mapped to chromosome 6BS in both tetraploid and hexaploid wheat (Mesfin et al. 1999; Chee et al, 2001). Thus, breeding for high grain protein content is

complex but may be more economical for wheat producers than raising protein by applying nitrogen fertilizer or choosing favorable wheat-growing conditions.

2.4.2.4 Composition of Protein

Wheat grain proteins can be divided into metabolic proteins and storage proteins, non-gluten and gluten, respectively (Shewry, 2003). Storage proteins (gluten) have been reported to have large effects on grain hardness and dough rheological properties (Dubreil et al. 1998). Storage proteins are crucial for forming the strong, cohesive dough which is suitable for producing a great diversity of food products, such as breads, noodles, pasta, cookies, cakes, pastries and many other foods (Weegels et al. 1996, Day et al. 2006). Wheat grain proteins can also be classified into albumins (water soluble), globulins (salt soluble), and prolamins (alcohol soluble) and glutelins (soluble in dilute acid or alkali) (Shewry et al. 1986; Shewry and Tatham, 1990) (Figure 3). Generally, albumins and globulins do not influence the rheological properties of wheat dough but do influence the processing properties of wheat products (Damodaran, 1996). The largest portion of wheat grain protein consists of prolamin storage proteins, which are classified into alcohol-soluble gliadins and alcohol-insoluble glutenins (Shewry and Tatham, 1990).

Glutenins are known as the biggest polymers in nature (Shewry and Halford, 2002). Glutenins consist of polypeptides aggregated by disulfide bonds (Shewry and Tatham, 1990; Singh and MacRitchie, 2001). Glutenins have high levels of glutamine and proline with low levels of charged amino acids (Goesaert et al. 2005), and appear to be largely responsible for gluten elasticity (MacRitchie, 1992; Wieser, 2007). Intermolecular disulfide bonding occurs toward the end of the chains on polymeric proteins, so in effect, the glutenin molecule is linear (Schofield, 1994; Gianibelli et al. 2001). The glutenin tertiary structure is thought to be one containing repetitive β-turns, which form a β-spiral structure (Tatham et al. 1985). This type of structure is stabilized by hydrogen bonding, and may explain the elastic nature of glutenin (Tatham et al. 1985). The glutenins are protein aggregates of high molecular weight

(HMW) and low molecular weight (LMW) glutenin proteins, which are stabilized by intermolecular disulfide bonds, hydrophobic interactions, and other forces (Gianibelli et al. 2001). The wheat gluten HMW-GS appear to be largely responsible for the elastic behavior of dough. They are major determinants of gluten elasticity, but are a small portion (5-10%) of total grain protein (Gianibelli et al. 2001; Wieser, 2007). HMW-GS affect gluten viscoelastic properties through their major effect on determining the size distribution of glutenin (MacRitchie and Lafiandra, 1997). There is evidence that gluten containing HMW-GS performs better in the bread-making process (Shewry et al. 1992). Motalebi et al. (2007) examined the positive relationship between HMW variation and pasta quality. The LMW-GS constitute the majority of wheat grain proteins, accounting for 60% of the total protein in the mature seed (Payne et al.1987; Gupta et al. 1989; Ciaffi et al. 1999, Wrigley 1996). The LMW-GS were reported to be highly correlated with gluten strength (Du Cros, 1987). It was reported that LMW-GS are important components of the giant gluten polymers that confer dough elasticity and extensibility (Wrigley, 1996).

Gliadins are a mixture of monomeric polypeptides (Sapirstein and Fu, 1998). Glutenins and gliadins have very similar amino acid composition, thus gliadins have a high level of proline and glutamine and have a low level of charged amino acids (Shewry, 2003). Gliadins can be classified into four groups of α, β, γ and ω on the basis of molecular mobility at low pH in acid polyacrylamide gel electrophoresis, all with similar amino acid compositions (Bushuk and Zillman, 1978; Tatham et al. 1990; Shewry et al. 1986). Gliadins may associate with other proteins or the glutenins through hydrophobic interactions and hydrogen bonds (Veraverbeke and Delcour, 2002; Bietz and Wall, 1980; Khatkar et al. 2002). Several studies have examined the relationship between gliadin proteins and dough rheological properties in wheat (Wrigley et al. 1981; Pogna et al. 1982; Dachkevitch et al. 1993). Hydrated gliadins have little elasticity and less cohesiveness than glutenins, but mainly contribute to the viscosity and extensibility of wheat dough (Wieser, 2007).

The genes, which are responsible for encoding gliadins and glutenin subunits, are located on several complex loci on the homeologous chromosomes 1 and 6 (Joppa et

al. 1983). Each of these homologous chromosomes consists of several tightly linked genes. HMW-GS are encoded by unique *Glu-1* loci (*Glu-A1*, *Glu-B1*, and *Glu-D1*) on the long arm of homeologous group 1 chromosomes. Each *Glu-1* locus has two linked genes designed as x-type (higher molecular weight) and y-type (lower molecular weight) based on differences in molecular weight, number of cysteine amino acid and repetitive motifs. A multigene family, *Glu-3* loci (*Glu-A3, Glu-B3,* and *Glu-D3*), which is responsible for coding LMW-GS, are located on the homeologous group 1 chromosomes (Gupta and Shepherd, 1990a, 1990b). However, LMW-GS encoded at the *Glu-B3* locus are important for good pasta quality (Ciaffi et al. 1991; Brites and Carrillio, 2001). The genes coding for LMW-GS also appear on the short arm of chromosomes 6A, 6B and 6D (Payne, 1987). LMW-GS can be classified into two patterns, LMW-1 and LMW-2, and explain a large part of the quality differences among durum wheat genotypes. Wheat cultivars carrying the LMW-2 glutenin subunits have stronger gluten than cultivars possessing LMW-1 (D'Ovidio and Masci, 2004; D'Ovidio, 1993; Vazquez et al. 1996). Although most recent durum wheat cultivars express the LMW-2 pattern, there is still considerable variation in gluten strength (Rao, 2008).

Genes coding for α and β gliadins are clustered at homeologous loci *Gli-A2* and *Gli-B2,* on the short arms of the group 6 chromosomes (Joppa et al. 1983). The genes coding for the γ and ω gliadins are clustered at *Gli-A1* and *Gli-B1* on the short arms of the chromosomes 1A and 1B (Troccoli et al. 2000). Gliadins also have higher allelic polymorphism than glutenins (Metakovsky and Branlard, 1998). The *Gli-B1* loci that encode γ- and ω-gliadins are tightly linked to the *Glu-B3* locus (Brown and Flavell, 1981).

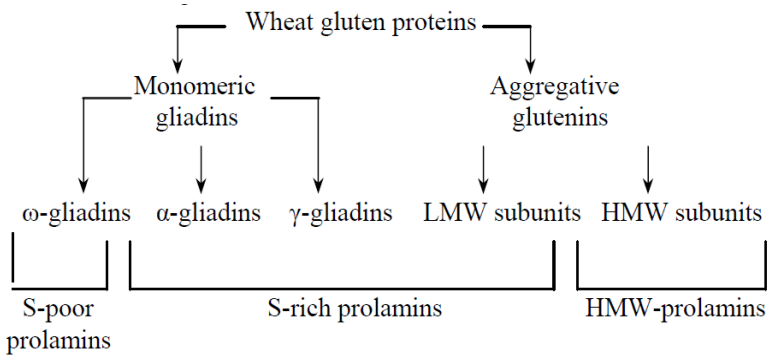

Figure 3: Traditional classification of gluten proteins (Shewry et al. 1986; Shewry and Tatham, 1990).

2.4.2.5 Gluten Strength

Gluten quality is a quality factor that influences pasta-cooking quality. Grain protein content and gluten quality both have been studied (Pagnotta et al. 2005), and are considered as the most important factors for determining the cooking quality of pasta products (Dexter and Matsuo, 1980; D'Egidio et al. 1990), and affecting the rheological properties of semolina dough (Payne et al. 1984; Pogna et al. 1994). Structural properties of gluten proteins have been studied to provide a basis for regulating and improving wheat end-use quality (Shewry et al. 1995; Shewry and Halford, 2002). Gluten is a rubbery mass obtained by washing wheat flour with water, while removing starch, non-starchy polysaccharides and water-soluble constituents. Gluten is a complex mixture consisting of two groups of proteins: the gliadins and glutenins (of 80–85%), associated with lipids (5%) and most of starch and non-starch carbohydrates (Wieser, 2007). The gliadins and glutenins constitute a majority of the total grain protein, and confer elasticity and extensibility properties to the dough (Shewry et al. 1995; Feillet, 1988; Shewry and Halford, 2002). In addition, the degree of sulfur bonding between gliadin and glutenin proteins determines the viscoelasticity of the gluten (Shewry et al. 1995).

Khatkar and Schofield (1997) suggested that gliadins and glutenins generally contribute to dough viscosity and dough elasticity, respectively. Wheat quality depends on the ratio of glutenin to gliadin proteins (Sapirstein and Fu, 1998), and the amount and size distribution of glutenin proteins (Gupta et al. 1993; Johansson et al.

2001). A higher glutenin-to-gliadin ratio leads to harder and stronger noodles. An increased resistance to extension, and a decrease in dough extensibility are important properties for superior pasta cooking quality (Feillet, 1988; Uthayakumaran et al. 1999). Gluten incorporation can increase tensile strength and hardness of cooked noodles (Park and Baik, 2009). In addition, the glutenin-to-gliadin ratio has an effect on wheat dough properties and loaf quality (Pechanek et al. 1997; Wieser and Kieffer, 2001).

Strong gluten proteins show good cooking quality based on their high elastic recoveries. In contrast, weak gluten proteins reflect poorer cooking quality because of their low elastic recoveries (Feillet, 1988). The intermolecular interaction between gluten proteins, and between protein and non-protein, forms various aggregates (Alamri et al. 2009). During cooking, water is prevented from penetrating into pasta by gluten proteins with high hydrophobicity so as to prevent swelling, surface disintegration, and pasta stickiness (Feillet, 1988). Excessive swelling of starch granules upon cooking can disrupt the integrity of the gluten protein network to a great extent, thereby reducing the cohesiveness of cooked noodles. The breaking length of cooked noodles was increased with a high glutenin-to-gliadin ratio (Zhang et al. 2011), because heat treatment accelerates the formation of crowded aggregates by LMW-GS (Feillet et al. 1989). Generally, durum cultivars with high levels of residue proteins and high glutenin-to-gliadin ratios exhibit good cooking quality.

Empirical information on rheological properties of dough can be obtained from instruments, such as the farinograph, mixograph, extensograph, alveograph, as well as glutograph. These methods are all recognized as physical indicators of gluten strength (Alamri et al. 2009). The glutograph is less used to estimate rheological properties of dough, but can be used for evaluating quality differences of the gluten by measuring the stretching and elastic properties of wet gluten (Alamri et al. 2009). The glutograph is suitable for large scale breeding programs, providing a rapid test compared to other methods (Sietz, 1987). Different values can be obtained from the glutograph profile such as the shear time (800 BU) and relaxation (after 10 seconds), which are related to the extension and the elasticity of the sample (Alamri et al. 2009).

2.5 Research Objectives and Questions

Sm1 has been transferred to durum wheat from common wheat (Clarke et al. 2010), but despite continuous efforts, durum wheat genotypes resistant to the orange wheat blossom midge (OWBM) have not been commercialized (as of February 2013), because they have not met the strict end-use quality requirements for the Canada Western Amber Durum (CWAD) wheat class. In most cases, durum wheat lines carrying *Sm1* have reduced grain protein content, and many exhibit reduced seed weight, low test weight, and reduced gluten strength. This could be due to genetic linkage of *Sm1* with genes that reduce end-use quality, or is the result of pleiotropic effects of *Sm1* directly on end-use quality. Currently, the size of the *Sm1* introgression into durum wheat is unknown, and efforts to map the size of the introgression are needed to reduce the linkage drag associated with *Sm1*. Also there is no published information available on the direct effects of *Sm1* on durum wheat quality.

As such, the following research questions need to be addressed:

a) Does the presence of *Sm1* impact the end-use quality properties of durum wheat?

b) What is the size of the *Sm1* introgression in durum wheat?

Given these research questions, the major objectives of this research will be to:

a) Evaluate the end-use quality of recombinant inbred lines segregating for *Sm1*;

b) Map the introgression of *Sm1* in a population of recombinant inbred lines segregating for *Sm1*.

2.6 Hypothesis

The main hypothesis of this research is that the presence of *Sm1* does not influence the end-use grain quality of durum wheat in the absence of OWBM damage.

3.0 Materials and Methods

3.1 Genetic Population

For this study, a durum wheat population derived from the cross “CDC Verona/ DT780” was utilized. CDC Verona (Pozniak et al. 2009) is spring durum wheat that lacks the *Sm1* gene, and is susceptible to the OWBM. DT780 is a DH (double haploid) breeding line developed by Dr. John Clarke, Agriculture and Agri-Food Canada, which carries the *Sm1* gene derived from the hexaploid winter wheat cultivar 'Caldwell' (Thomas et al. 2005). The pedigree of DT780 is KYLE//9560A-138/94B27-BR1C/3/Napoleon/4/AC Navigator/5/Strongfield. This population was designated as D05M.80, and a total of 122 $F_{5:9}$ recombinant inbred lines are available and were used for this study. The D05M.80 population, along with parents of the mapping population and appropriate check cultivars were phenotyped previously by assessing midge damage in replicated plots conducted in environments with high midge pressure (Dr. Curtis Pozniak, unpublished results; Appendix 1, 2 and 3).

3.2 Field Trials

In 2009, the D05M.80 population was grown along with parents and appropriate check cultivars (Table 2) in two replicated trials at the Kernen Crop Research Farm (KCRF), University of Saskatchewan, at Saskatoon, SK, and at the Agriculture and Agri-Food Canada Research Farm in Indian Head, SK, Canada. The experiment was repeated in 2010 at the KCRF, using the same experimental design. The experimental design was a randomized complete block design. The plot area was 0.743 square meters (2.43 meter long by 0.305 meter wide) for Kernen 2009 and 2.76 square meters (3 meter long by 0.92 meter wide) for Indian Head 2009 and 2.43 square meters (a single 2.43 meter row) for Kernen 2010. The seeding rate was 300 seeds/m^2 for each plot. At physiological maturity, plants were harvested with a small plot combine and grain samples were cleaned with a Carter-Day Dockage Tester

(USDA; Carter Day International, Inc) (screen size: 1.19 centimeter wide and 0.238 centimeter long) with aspiration channel. This sieving removed heavily midge-damaged kernels prior to quality assessments.

Table 2: Check cultivars used in field experiments in 2009 and 2010.

Check	**Ploidy**	***Sm1***
Goodeve	Hexaploid	Yes
Waskada	Hexaploid	No
AC Avonlea	Tetraploid	No
AC Morse	Tetraploid	No
AC Navigator	Tetraploid	No
Brigade	Tetraploid	No
Commander	Tetraploid	No
Eurostar	Tetraploid	No
Strongfield	Tetraploid	No
CDC Verona	Tetraploid	No
DT780	Tetraploid	Yes

3.3 Trait Evaluations

For field trials, days to heading (Zadoks growth stage 55; Zadoks et al. 1974) was recorded for each plot. Plant height of all plots was measured at maturity; plots were harvested with a plot combine; and the grain weight per plot was measured. Yield was converted to kg/ha based on the total area harvested prior to statistical analysis. Thousand-kernel weight was measured by weighing 1000 seeds, following counting with an electronic seed counter (Agriculex Inc., Guelph, Ontario, Canada). Test weight (kg/hl) was estimated determined on a plot basis by weighing a fixed volume of grain samples and using a Schopper chondrometer based on the AACC method 55-10 (AACC, 2000).

Seed samples (60 g) from each plot were ground into whole wheat meal using

Udy Cyclone sample mill (Udy Corporation., Fort Collins, CO) fitted with a 1-mm screen/mesh. Moisture content of ground meal was obtained by heating approximately 3 g samples for 65 minutes/130°C, in accordance with standard AACC method 44-15A (AACC, 2000) on a random set of 20 samples from each trial. The average moisture content of these 20 samples was used as an estimate of the moisture content of the remaining samples. All chemical grain quality assessments are reported on 14% moisture basis. Whole meal samples were used for determining grain protein content (%) and yellow pigment content (mg/kg), which was estimated by using a Foss (NIR System) Model 6500 (FOSS Inc., Silver Spring, MD, USA) spectrophotometer that was calibrated against a set of reference samples.

The Hagberg Falling Number (Falling Number 1700 System, Perten Instruments, Sweden) test was evaluated on a plot basis using AACC Method 56-81B (AACC, 2000). Falling number was expressed in seconds and provided a measurement of starch viscosity by heating viscometer tubes with ~6.6 g sample (based on 14% moisture basis) and 25 ml distilled water.

Gluten strength was assessed on a plot basis using the Glutomatic (AACC Method 38-12A; AACC, 2000) and Glutograph-E (AACC Method 54-40A; AACC, 2000). Approximately 9.3 g of meal (based on 14% moisture basis) was washed with a 2% sodium chloride solution in a Gluten washer for 5 min (2 min with coarse sieve, 3 min with fine sieve), two pieces of wet gluten were separately transferred into the Glutomatic and Glutograph after a 5-minute resting period. Wet gluten was expressed as a percentage of the sample weight. For the gluten index, washed gluten was centrifuged at a speed of 6000±5rpm in the Glutomatic. The gluten index (GI, %) was estimated by weighing the wet gluten remaining on the sieve relative to the total wet gluten content. The Glutograph instrument measured the dough stretching time (seconds) to a constant deflection in seconds after reaching a consistency of 800 Brabender units (BU) and relaxation after 10 seconds of stretching the washed gluten. Stretching time is a measure of gluten strength, and relaxation is a measure of dough extensibility.

3.4 Evaluation of Midge Damaged Seeds

At maturity, 15 spikes randomly picked from each plot were collected by hand from upper canopy stems at KCRF in 2010 to assess midge pressure. These spikes were air-dried and stored to avoid breakage. Ten spikes were randomly selected from the 15 harvested spikes, and manually threshed. All seeds, including extremely damaged and very small ones, were retained and stored in a labeled envelope. The seeds were classified into four categories (Category 1=undamaged seeds, Category 2=slightly damaged seeds, Category 3=badly damaged seeds, and Category 4=extremely damaged seeds), as shown in Figure 4, and the number in each category for each spike was expressed as a percentage of the total number of seeds. Seeds classified as category 1 were plump, with no rupture, line or mark and no distortion on either the back or side of the kernel; seeds in category 2 were slightly ruptured, and had few lines or marks on either the back or side of the kernel, or were slightly distorted; seeds in category 3 were more severely damaged than those in category 2, with a distinct rupture or white line or mark on the back or side of the kernel, or distinctly distorted; seeds in category 4 were light, small and shriveled, as a result of extreme damage by OWBM (Figure 4). Data from the 10 spikes were then averaged for each field plot prior to statistical analysis.

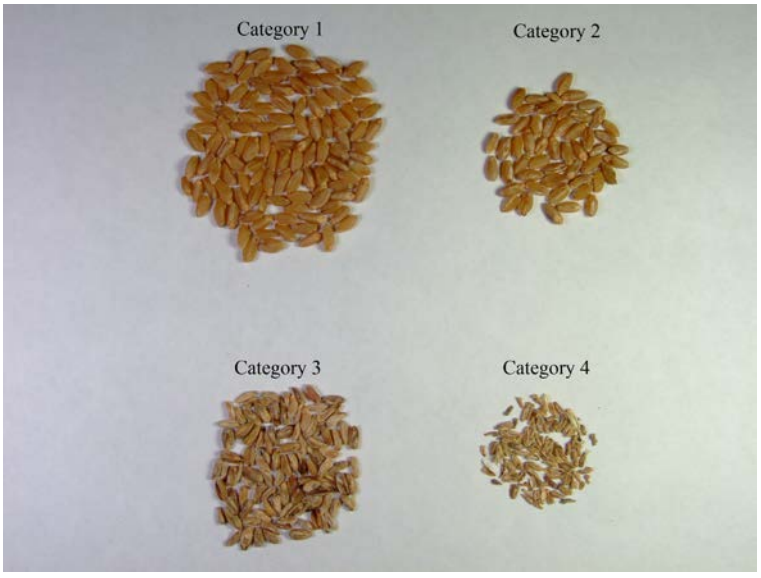

Figure 4: Classification of damaged seeds into four categories of damage.

3.5 Mapping the *Sm1* Introgression in Durum Wheat

To map the *Sm1* introgression, DNA markers known to reside on chromosome 2B near *Sm1* were used (Thomas et al. 2005). The majority of molecular markers assessed were microsatellite markers, which have been used extensively in wheat (Somers et al. 2004) and in several Canadian durum wheat populations (Pozniak et al. 2007; Singh et al. 2009). DNA was extracted from leaf tissue of DT780 and CDC Verona using a modified hexadecyltrimethyl-ammonium bromide (CTAB) method (Pozniak et al. 2007). Microsatellite markers (See section 3.5.1 below) and expressed sequence tag (EST) markers (see section 3.5.2 below) were first assessed for polymorphisms on extracted DNA from DT780 and CDC Verona, using capillary electrophoresis (ABI3100xl; Applied Biosystems) or single strand conformation polymorphism (SSCP) gels, respectively.

3.5.1 SSR Marker Analysis

In total, thirty-four microsatellite primers were selected for polymorphisms based on the results from the two parents, and evaluated for polymorphism in the whole population. PCR reactions were performed in 96-well PCR plates with 25 µl of reaction mixture. Reactions contained 50 ng/µl of genomic DNA, 2.5µl 10× PCR buffer (Gene Script), 1 µl dNTP (5 mM), 1 µl microsatellite primers (MF-2.5 um/µl and R-10 um/µl), 0.38µl the Universal dye-labeled M13 primer (sequence modified forward microsatellite primer; 5'-CACGACGTTGTAAAACGAC-3'), 0.25 µl of Taq DNA polymerase (Gene Script) and 18.87 µl autoclaved distilled water. The forward primer of each microsatellite marker pair was modified by incorporating the Universal M13 sequence to the 5' end during synthesis (Schuelke, 2000). The Universal M13 primer was labeled with fluorescent dyes (FAM, VIC, NED or PET). Temperature cycling was as follows: initial denaturation of 3 min at 94ºC, followed by three cycles of 94 ºC for 30 sec (denature), 45 sec for annealing with temperature touchdown from 62 (56) to 56 (50) ºC (three cycles×four; every three cycles decreased 2 ºC) and 72 ºC for 45 sec, and then followed by 33 cycles of 94 ºC, 51 ºC

for 45 sec and 72 ºC for 45 sec, followed by a final extension at 72 ºC for 10 min before cooling to 4 ºC.

Two methods were used for polymorphism detection. First, amplification products were resolved by electrophoresis in 1.0% agarose gels at low voltage (126 V) in 0.5× TBE buffer and then stained with ethidium bromide (0.5 µg/ml). The DNA banding patterns were visualized with UV light. If no polymorphisms were detected on agarose gels, capillary electrophoresis was performed where 1µl of diluted PCR products (dilution ratio: 1/10~1/2) was combined with 9.0 µl HiDi formamide (ABI, Foster City, California) and 0.08µl ROX size standard and run on a 36 cm capillary electrophoresis (ABI3100xl; Applied Biosystems), and processed with Applied Biosystem Data Collection Software version 2.0.

3.5.2 EST Marker Development and Analysis

Thirty-one EST markers (expressed sequence tag sequences) were designed based on rice and *Brachypodium distachyon* genomic information. These ESTs have been used for developing PCR-based DNA markers for saturation and mapping of chromosomal regions where the *Sm1* gene maybe located. Thomas et al. (2005) reported that *Sm1* is linked to *Xgwm210*, and that marker has been localized to wheat deletion BIN 2BS-4 (Figure 5). The 31 EST markers were selected for analysis because they have been previously localized to the same deletion BIN (http://wheat.pw.usda.gov/GG2/index.shtml). Available EST sequences were first blasted against the rice and *Brachypodium* genomic and coding sequence by using NCBI BLAST (GrainGenes 2.0; http://wheat.pw.usda.gov/GG2/index.shtml). Sequences were then aligned using AlignX (Vector NTI Advance 10.3; Invitrogen, Carlsbad, CA). After alignment, primers were designed to amplify the wheat sequence by selecting primer binding sites that were highly similar between all sequences. Polymorphic EST-designed primers were scored by using SSCP.

Single-stranded conformation polymorphism (SSCP) is a method of Nucleotide sequence analysis of DNA fragments, which is used to assess genetic variability

among related viral strains. PCR products of the mapping population were first amplified by PCR reactions, which were performed in a 384-well PCR plate with 15µl of reaction mixture. Reactions contained 50 ng/µl of genomic DNA, 1.5 µl 10× PCR buffer (Gene Script), 0.6 µl dNTP (5mM), 0.8 µl microsatellite primers (MF: 10 um/µl and R: 10 um/µl), 0.15 µl of Taq DNA polymerase (Gene Script) and 11.25 µl autoclaved distilled water. Temperature cycling was: initial denaturation of 5 min at 94 ºC, followed by 38 cycles of 94 ºC for 30 sec, 58 ºC for 30 sec and 72 ºC for 1 min, and then followed by a final extension at 72 ºC for 10 min before cooling to 4 ºC.

Glass plates for gel construction were first cleaned with dish soap, rinsed thoroughly with distilled water and rinsed with 95% ethanol and air-dried. Sigmacote was applied to the rear plate for 10 min and the plate was wiped with 95% ethanol with Kimwipes three times. A binding solution (1984 µl 95% ethanol, 10 µl Acetic Acid and 6 µl Silane) was applied to the front plate for 5 min and fully wiped with 95% ethanol three times. After plate preparation, the two plates were tightly assembled in a gel box with two side clamps and held together by a stand. Gels were prepared using 100 ml 0.6X MDE gel (Lonza, Rockland, ME, USA) with 30 ml 2×MDE gel solution, 6 ml 10× TBE Buffer and 64 ml distilled water. MDE gel solution was filtered with a 40 micron syringe filter and degassed with a vacuum flask. Just before casting the gel, 50 µl of TEMED and 100 µl of 25% APS (frozen) were added into the MDE gel solution. The MDE gel solution was well mixed and then poured into the pre-prepared gel box.

PCR products were diluted (1:5) in a denaturing loading mix (4 µl of PCR products mixed with 20 µl of loading buffer containing 95 % formamide, 0.05 % bromophenol blue, and 0.05 % xylene cyanol), and heated at 95 ºC for 5 min, and then plunged immediately into ice to allow single strand folding. The fragments were resolved on the MDE gel run at room temperature for 17 h (6 W) using 0.6× TBE buffer. The Bio-Rad Sequi-Gen GT System (38×50 cm) was used for electrophoresis. Gels were visualized by silver staining using the following steps: fixed by agitating 20 min in a fixing solution (2 L; 1800 ml distilled water and 200 ml acetic acid); rinsing 3 times (2 min each) with distilled water; staining by agitating 30 min in a staining

solution (2 L distilled water, 2 g silver nitrate and 3 ml of 37 % formaldehyde); briefly rinsing (5 sec) in distilled water; agitation in 2 L developing solution (below 10 ºC, 4 L distilled water mix well with about 120 g sodium carbonate; 6 ml 37% formaldehyde and 800 ul sodium thiosulfate (10 mg/ml) were immediately added into the solution) for 5-10 min until some of the bands started showing; agitating the gel in a new 2 L developing solution until all of the targeted bands were visible; finally fixed by the previously reserved fixing solution for 3 min and rinsed with distilled water for two times (2 min each).

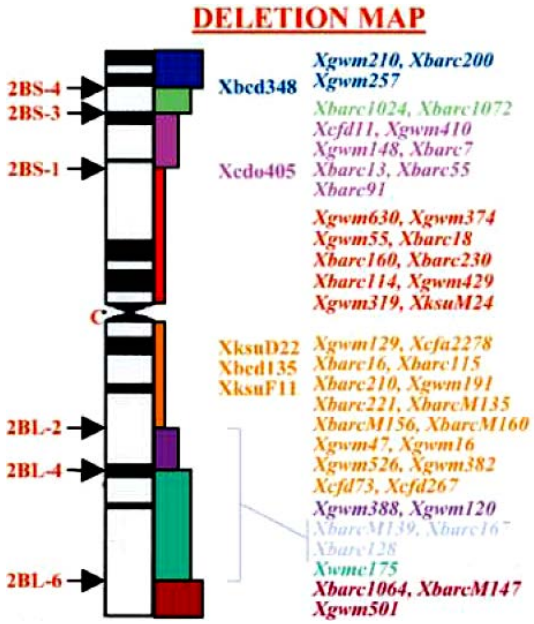

Figure 5: Current Assignment of molecular markers to deletion BINS on chromosome 2B (Sourdille et al. 2004)

3.5.3 DArT® Marker Analysis

Diversity Arrays Technology (DArT) is a marker technology that will simultaneously type several hundred polymorphic loci spread over a genome and has been used extensively in wheat (Akbari et al. 2006; Mantovani et al. 2008).

DNA samples of the mapping population were sent to a whole-genome profiling service laboratory (Diversity Arrays Technology Pty Ltd; http://www.diversityarrays.com/) for DArT marker analysis. Twenty-nine DArT markers were polymorphic on chromosome 2B, and mapped in the mapping

population. These polymorphic DArT markers were used to generate a chromosome 2B genetic map together with SSR and EST markers.

3.5.4 Genetic Map and QTL Analysis

A genetic map of chromosome 2BS was constructed using the software JoinMap®4 (van Ooijen and Voorrips, 2004). Markers displaying high frequencies of double recombination were removed before assembling the final map. The final genetic map was selected based on good marker alignment with the lowest frequencies of double recombination and comparison against other published maps (Thomas et al. 2005; Somers et al. 2004; Singh et al. 2009). The final map consisted of EST markers, DArT® markers (designated as 2B_wPt), and microsatellite markers (designated as *Xgwm* or *Xwmc*). *Sm1* was mapped as a Mendelian factor by classifying lines as either resistant or susceptible. Segregation of each polymorphic marker locus in the RIL population was analyzed for goodness of fit to 1:1 and 3:1 ratios using a Chi-square test (Strickberger, 1985) at $P>0.05$.

For QTL analysis, least square (LS) means of each trait in each environment and the mean phenotypic value of each trait across environments were used in the QTL analysis. The QTL analysis was performed using MapQTL®6 (Van Ooijen, 2009). One thousand permutations for each marker interval were used to determine LOD score at a genome-wide significance level of 0.05. Interval mapping was first performed to identify QTLs, and MQM mapping was subsequently performed by selecting marker cofactors. MapChart 2.2 (Voorrips, 2002) was used to visualize genetic linkage and QTL maps. The additive effect of each QTL was estimated as one-half the difference between marker classes.

3.6 Statistics Analysis

Analysis of variance (ANOVA) for all trait data (from 2009 and 2010) was performed using PROC MIXED of SAS (Version 9.2) (SAS Institute Incorporated, Cary, North Carolina, USA). Locations and replications were considered random

factors. Entry (11 check cultivars and D05M.80 population) and *Sm1* genotypes (*Sm1* "+/-") were considered fixed effects. Each location-year combination was treated as an environment. Analysis model assumptions were evaluated by testing the distribution of plot residuals, which tended to be non-normal. Normality of distribution ($P>0.05$, 5% significance level) was checked by using the Shapiro-Wilk test (Shapiro and Wilk, 1965). Traits showing heteroscedasticity of random variance estimates from the different environments were not combined by genotypes and locations. However, combined analysis across different environments was still performed using the PROC MIXED of SAS program as supplemental information.

SAS was used to estimate Pearson's correlation coefficients among measured traits of the mapping population from either two or three environments, excluding the checks. Contrast analysis was used to compare all traits among genotypes from the mapping population, using the *Sm1* genotype as the classification variable. Significant differences were determined using Fisher's LSD test at $\alpha=0.05$.

4.0 Results

4.1 Analyses of Agronomic and Quality Traits

ANOVA analyses were conducted for all measured agronomic and quality traits by individual environment, and across environments and are presented in Table 3-7. For individual environments, the effects of replication were not significant for the observed traits, but the effects of *Sm1* were significant for most traits (Table 3, 4 and 5). For the ANOVA, the effects of each entry were portioned into that genotypic variation which was explained by *Sm1* within the durum RIL population, and the remaining variation among the RILs and check cultivars. The ANOVA for Kernen 2009 revealed highly significant effects of *Sm1* on most measured traits, with the exceptions of HD, PH, GPC, ST and RELAX (Table 3). At Kernen 2010, the ANOVA revealed significant effects of *Sm1* on most measured traits, with the exceptions of HD, PH, TWT and RELAX (Table 4). The agronomic data for Indian Head (2009) was incomplete, so only the quality data was analyzed (Table 5). Significant *Sm1* effects were observed on all the measured traits, except FN and WETG (Table 5). Variation not explained by *Sm1* was significant for most of the observed traits in individual environments, with the exception of WETG at Indian Head 2009 and ST at Kernen 2009 (Table 3 and 5). This was expected because several of the check cultivars were hexaploid wheat, which have a unique quality profile relative to durum wheat (Table 2, Section 3.2).

Table 3: Variance estimates for random effects and F-tests for fixed effects from analysis of variance (ANOVA) of heading date (HD), plant height (PH), yield (YLD), test weight (TWT), thousand-kernel weight (TKW), falling number (FN), grain protein content (GPC), yellow pigment (YP), wet gluten content (WETG), gluten index (GI), stretching time (ST) and relaxation (RELAX) (14% moisture basis) for Kernen 2009.

Source	**HD (day)**	**PH (cm)**	**YLD (kg/ha)**	**TWT (kg/hl)**	**TKW (g)**	**FN (sec)**	**GPC (%)**	**YP (mg/kg)**	**WETG (%)**	**GI (%)**	**ST (sec)**	**RELAX (BU)**
Random Effects (variance estimate)												
Replication	0	3.14	56554	0	0	80.5	0.0197	0	0	2.63	15.2	106
Residual	0.861	14.1	420724	0.256	3.28	416	1.02	0.0777	13.8	126	236	787
	***	***	***	***	***	***	***	***	***	***	***	***
Fixed Effects (F value)												
***Sm1*Genotype**	0.40	2.39	17.0	70.4	47.7	22.8	0	119	11.8	33.3	3.62	1.93
			***	***	***	***		***	***	***	(0.0596)	
***Sm1*Genotype (Entry)**	2.70	3.20	1.57	6.38	4.79	3.53	1.70	10.7	1.78	4.27	1.31	1.74**
	***	***	**	***	***	***	**	***	***	***		

*p<0.05; **p<0.01; ***p<0.001

Table 4: Variance estimates for random effects and F-tests for fixed effects from analysis of variance (ANOVA) of heading date (HD), plant height (PH), yield (YLD), test weight (TWT), thousand-kernel weight (TKW), falling number (FN), grain protein content (GPC), yellow pigment (YP), wet gluten content (WETG), gluten index (GI), stretching time (ST) and relaxation (RELAX) (14% moisture basis) for Kernen 2010.

Source	HD (day)	PH (cm)	YLD (kg/ha)	TWT (kg/hl)	TKW (g)	FN (sec)	GPC (%)	YP (mg/kg)	WETG (%)	GI (%)	ST (sec)	RELAX (BU)
Random Effects (variance estimate)												
Replication	0.0236	4.70	58652	0.000128	0.0323	0	0.00652	0.00119	0.0874	0	0	1.49
Residual	0.481	40.8	198106	0.413	3.22	159	0.220	0.0885	2.27	17.8	11.4	109
	***	***	***	***	***	***	***	***	***	***	***	***
Fixed Effects (F value)												
***Sm1*Genotype**	3.68 (0.0574)	0.93	331 ***	2.4	122 ***	69.8 ***	299 ***	16.5 ***	133 ***	473 ***	71.1 ***	0.79
***Sm1*Genotype (Entry)**	4.18 ***	2.23 ***	2.58 ***	7.71 ***	4.13 ***	6.21 ***	5.73 ***	7.94 ***	9.05 ***	13.4 ***	7.34 ***	6.95 ***

*p<0.05; **p<0.01; ***p<0.001

Table 5: Variance estimates for random effects and F-tests for fixed effects from analysis of variance (ANOVA) of falling number (FN), grain protein content (GPC), yellow pigment (YP), wet gluten content (WETG), gluten index (GI), stretching time (ST) and relaxation (RELAX) (14% moisture basis) for Indian Head 2009.

Source	FN (sec)	GPC (%)	YP (mg/kg)	WETG (%)	GI (%)	ST (sec)	RELAX (BU)
Random Effects (variance estimate)							
Replication	64.6	0.123	0.0403	5.75	0	0	0.862
Residual	677	0.628	0.0301	159	83.5	22.6	224
	***	***	***	***	***	***	***
Fixed Effects (F value)							
***Sm1*Genotype**	2.99	13.8	301	0.87	58.4	44.2	8.94
		***	***		***	***	**
***Sm1*Genotype (Entry)**	2.92	1.70	20.9	1.03	8.86	5.83	4.05
	***	**	***		***	***	***

*p<0.05; **p<0.01; ***p<0.001

Table 6: Combined ANOVA for grain quality traits over two environments (Kernen 2009 and Kernen 2010). Variance estimates for random effects and F-tests for fixed effects from analysis of variance (ANOVA) of heading date (HD), plant height (PH), yield (YLD), test weight (TWT), and thousand-kernel weight (TKW).

Source	HD (day)	PH (cm)	YLD (kg/ha)	TWT (kg/hl)	TKW (g)
Random Effects (variance estimate)					
Environment	0.238	0	1351046	36.1	0
Environment (Replication)	0.00819	3.48	57496	0	0.00164
Environment*Entry	0.0914	5.42 *	134159 ***	0.922 ***	2.74 ***
Residual	0.675 ***	27.4 ***	307846 ***	0.335 ***	3.27 ***
Fixed Effects (F value)					
***Sm1*Genotype**	2.14	1.86	100.6 ***	2.41	59.7 ***
***Sm1*Genotype (Entry)**	4.05 ***	2.55 ***	1.23	1.26	2.34 ***

*p<0.05; **p<0.01; ***p<0.001

Table 7: Combined ANOVA for grain quality traits over three environments (Indian Head 2009, Kernen 2009 and Kernen 2010). Variance estimates for random effects and F-tests for fixed effects from analysis of variance (ANOVA) of falling number (FN), grain protein content (GPC), yellow pigment (YP), wet gluten content (WETG), gluten index (GI), stretching time (ST) and relaxation (RELAX) (14% moisture basis).

Source	FN (sec)	GPC (%)	YP (mg/kg)	GI (%)	WETG (%)	ST (sec)	RELAX (BU)
Random Effects (variance estimate)							
Environment	29268	11.3	1.24	50.5	82.2	2.98	0
Environment (Replication)	47.7	0.0496	0.0137	0.597	1.95	4.98	26.6
Environment*Entry	264 ***	0.191 ***	0.0801 ***	62.3 ***	2.18	9.14	118.8 ***
Residual	418 ***	0.621 ***	0.0655 ***	76.0 ***	58.7 ***	89.6 ***	372 ***
Fixed Effects (F value)							
***Sm1*Genotype**	0.50	40.4 ***	78.1 ***	85.4 ***	1.42	24.7 ***	4.73 *
***Sm1*Genotype (Entry)**	2.87 ***	2.33 ***	7.66 ***	5.58 ***	1.38 *	2.84 ***	2.97 ***

*p<0.05; **p<0.01; ***p<0.001

A combined data analysis over two environments was conducted for HD, PH, YLD, TWT, TKW (Table 6); and a combined analysis over three environments was conducted for end-use quality traits such as FN, GPC, YP, WETG, GI, ST and RELAX (Table 7). Similar to the individual environments, the entry variation was portioned into variation within and among *Sm1* carriers. The effects of individual environment and replication nested within environment were not significant for any of the observed traits (Table 6 and 7). With the combined data set, the effects of *Sm1* within the mapping population were statistically significant for YLD, TKW, GPC, YP, GI, ST and RELAX (Table 6 and 7). The entry nested within *Sm1* genotype was highly significant ($P<0.01$) for almost all traits, with the exception of YLD and TWT (Table 6 and 7).

4.2 Pearson's Correlation Analysis of Agronomic and Grain Quality Traits

Table 8 presents the correlation coefficients between FN, GPC, YP, WETG, GI, ST and RELAX across three environments. The correlation coefficients between all pairs of traits across two environments (Kernen 2009 and Kernen 2010) are presented in Table 9. Significant correlations were observed for nearly all trait combinations (Table 8 and 9). The strongest positive and negative correlations were observed between FN and TWT ($r=0.945$, $P<0.001$; Table 9) and FN and GPC ($r=-0.897$, $P<0.001$, Table 8; $r=-0.869$, $P<0.001$, Table 9), respectively. The GPC was also negatively correlated with YLD ($r=-0.669$, $P<0.001$, Table 9) and TWT ($r=-0.887$, $P<0.001$, Table 9). YLD was positively correlated with TWT ($r=0.714$, $P<0.001$, Table 9), and FN ($r=0.709$, $P<0.001$, Table 9). Both YLD and TWT were negatively correlated ($r=-0.556$, $r=-0.728$, $p<0.001$, respectively, Table 9) with YP. FN was negatively correlated with YP ($r=-0.766$, $P<0.001$, Table 8; $r=-0.688$, $P<0.001$, Table 9). However, GPC was positively correlated with YP ($r=0.747$, $P<0.001$, Table 8; $r=0.617$, $P<0.001$, Table 9).

WETG was strongly correlated with GPC (r=0.754, P<0.001; Table 8; r=0.937, P<0.001, Table 9), and YP (r=0.558, P<0.001, Table 8; r=0.556, P<0.001, Table 9). On the contrary, strong negative correlations were obtained between WETG and TWT (r=-0.804, p<0.001, Table 9), WETG and FN (r=-0.650, p<0.001, Table 8; r=-0.782, P<0.001, Table 9) and WETG and YLD (r=-0. 564, P<0.001, Table 9). GI was positively correlated to ST (r=0.560, p<0.001, Table 8; r=0.523, p<0.001, Table 9). Strong gluten strength properties were accompanied by lower relaxation, since ST and RELAX showed a negative correlation (r=-0.797, p<0.001, Table 8; r=-0.790, p<0.001, Table 9).

Table 8: Pearson's correlation coefficients between falling number (FN), grain protein content (GPC), yellow pigment (YP), wet gluten content (WETG), gluten index (GI), stretching time (ST) and relaxation (RELAX) (14% moisture basis) for the CDC Verona/DT780 population from three environments (Indian Head 2009, Kernen 2009 and Kernen 2010).

	GPC	YP	GI	WETG	ST	RELAX
FN	-0.897***	-0.766***	0.316***	-0.650***	0.183***	0.057
GPC		0.747***	-0.351***	0.754***	-0.185***	-0.033
YP			-0.238***	0.558***	-0.119***	-0.098**
GI				-0.301***	0.560***	-0.481***
WETG					-0.214***	0.045
ST						-0.797***

*p<0.05; **p<0.01; ***p<0.001

Table 9: Pearson's correlation coefficients between yield (YLD), heading date (HD), plant height (PH), test weight (TWT), thousand-kernel weight (TKW), falling number (FN), grain protein content (GPC), yellow pigment (YP), wet gluten content (WETG), gluten index (GI), stretching time (ST) and relaxation (RELAX) (14% moisture basis) for the CDC Verona/DT780 population from two environments (Kernen 2009 and Kernen 2010).

	PH	YLD	TWT	TKW	FN	GPC	YP	GI	WETG	ST	RELAX
HD	0.456***	0.256***	0.262***	0.209***	0.251***	-0.208***	-0.218***	-0.002	-0.144**	-0.018	0.115*
PH		0.122**	-0.010*	0.205***	-0.115*	0.188***	-0.006	-0.123**	0.209***	-0.083	0.101*
YLD			0.714***	-0.024	0.709***	-0.669***	-0.556***	0.027	-0.564***	0.047	0.098*
TWT				-0.004	0.945***	-0.887***	-0.728***	0.287***	-0.804***	0.170***	0.084
TKW					-0.078	0.114	-0.224***	0.014	0.073	0.042	0.074
FN						-0.869***	-0.688***	0.292***	-0.782***	0.197***	0.042
GPC							0.617***	-0.309***	0.937***	-0.204***	-0.040
YP								-0.158***	0.556***	-0.112	-0.100*
GI									-0.452***	0.523***	-0.431***
WETG										-0.330***	0.104*
ST											-0.790***

*p<0.05; **p<0.01; ***p<0.001

4.3 Evaluation of Midge Damage on Seeds

In 2010, high midge pressure was observed at the Kernen Crop Research Farm. As such, individual lines were assessed for proportion of midge damaged seed. In the mapping population, midge damaged seeds had values ranging from 25.3 to 73.9 % in Category 1; from 7.55 to 27.1 % in Category 2; from 12.5 to 50.6 % in Category 3 and from 0 to 22.7 % in Category 4 (Appendix 1). Transgressive segregation was evident for damaged seeds, and some lines carrying *Sm1* within RIL lines showed greater resistant properties than check cultivars carrying *Sm1* (Appendix 1).

Table 10: Variance estimates for random effects and F-tests for fixed effects from analysis of variance (ANOVA) of damaged seeds (Category 1=undamaged seeds, Category 2=slightly damaged seeds, Category 3=badly damaged seeds and Category 4=extremely damaged seeds) of the CDC Verona/DT780 population at Kernen 2010, using PROC MIXED.

Source	Category 1	Category 2	Category 3	Category 4
Random Effects (variance estimate)				
Replication	2.78	1.62	0	0.674
Residual	66.6***	15.2***	29.4***	15.5***
Fixed Effects (F value)				
***Sm1*Genotype**	77.1***	40.9***	14.3***	375***
***Sm1*Genotype (Entry)**	2.23***	1.49*	2.54***	1.41*

*p<0.05; **p<0.01; ***p<0.001

Table 11: Percentages of damaged seeds (Category 1=undamaged seeds, Category 2=slightly damaged seeds, Category 3=badly damaged seeds and Category 4=extremely damaged seeds) in each check and the mapping population.

Accessions	*Sm1*"-"/*Sm1* "+" (S/R)	Category 1 (%)	Category 2 (%)	Category 3 (%)	Category 4 (%)
Goodeve	R	61ab	13bcdef	16e	10bc
Waskada	S	50abcd	21a	16e	13abc
AC Avonlea	S	57abc	11def	23cde	10bc
AC Morse	S	40de	18abcd	30abc	12abc
AC Navigator	S	40de	15abcdef	36a	9bc
Bridage	S	40de	20ab	29abc	10bc
Commander	S	32e	19abc	34ab	16ab
Eurostar	S	51abcd	10f	25bce	14abc
Strongfield	S	50abcd	18abcde	24bce	8c
CDC Verona	S	39de	12cdef	30abc	18a
DT780	R	46bcde	14abcdef	30abc	11bc
Sm1"-"	S	47cd	13ef	27bd	14ab
Sm1"+"	R	58a	17abc	24c	2d
LSD $_{0.05}$ ***(Sm1"-" vs Sm1"+")***		2.46	1.17	1.63	1.18

For each column, means followed by the same letter are not significantly different (p=0.05).

As shown in Table 10, the ANOVA for damaged seed revealed highly significant effects of *Sm1* on all categories. The contrast analysis detected significant differences

in the proportion of seeds in each category between *Sm1* carriers and non-carriers of the mapping population (Table 11). However, the ANOVA also revealed a significant *Sm1* genotype (entry) interaction, suggesting that factors other than *Sm1* were strongly influencing seed classification (Table 10).

'DT780' and 'CDC Verona' exhibited no significant differences for the first three grain damage categories (Table 11). However, 'DT780', carrying *Sm1*, had a smaller proportion (11%) of seeds in category 4 (Table 11). 'Goodeve', a hexaploid wheat check that carries *Sm1*, had a significantly higher proportion of seeds in category 1, and significant lower proportion of seeds in category 3 compared to 'DT780' (Table 11). Likewise, *Sm1* non-carrier, 'Waskada' is known to deter egg-laying by the OBWM, showed a high proportion of seeds classified into category 1 (Table 11). The durum wheat checks could be classified into two categories. 'AC Avonlea', 'Eurostar', and 'Strongfield' had a higher proportion of category 1 seeds, than AC Morse', 'AC Navigator', 'Commander', 'Brigade', and 'CDC Verona' (Table 11).

On average, *Sm1* carriers in the RIL mapping population had a significantly higher proportion of seeds in categories 1 and 2, and significantly lower proportion of seeds in categories 3 and 4, when compared to *Sm1* non-carriers (Table 11).

4.4 Effect of *Sm1* on Agronomic and End-use Grain Quality Traits

4.4.1 Heading Date (HD), Plant Height (PH), and Test Weight (TWT)

The LS means for HD, PH and TWT for the check cultivars and each of the lines from the mapping population are presented in Appendix 2. There were significant differences ($p<0.05$) between RIL lines for all three traits at Kernen in both 2009 and 2010. Transgressive segregation was evident, with RIL lines showing significantly greater and lower expression of HD, PH and TWT than either of 'DT780' and 'CDC Verona' (Appendix 2 and Figure 6).

In the mapping population, no significant differences were detected for *Sm1*, except YLD and TWT at Kernen 2009 and Kernen 2010, and YLD at Kernen 2010 (Table 3 and 4). The HD of all checks and the mapping population were similar in

both environments (Table 12). CWRS cultivars 'Waskada' and 'Goodeve' both headed earlier than other checks and the mapping population (Table 12). Compared to Kernen 2009, higher PH and earlier HD were observed at Kernen 2010 for most checks and in the mapping population with the exception of 'AC Avonlea' and 'Strongfield' (Table 12). In both environments, 'Commander' expressed short PH (Table 12), which was expected as 'Commander' carries the *Rht-B1b* dwarfing gene (Clarke et al. 2005).

Average TWT was significantly different between *Sm1* carriers and *Sm1* non-carriers at Kernen 2009, being 0.6 kg/hl less in *Sm1* carriers (Table 12). The *Sm1* carriers of the mapping population also showed, on average, lower TWT than their parents 'CDC Verona' and 'DT780' (Table 12). In addition, 'CDC Verona' revealed higher TWT than 'DT780' in both environments (Table 12). 'Waskada' and 'Goodeve', both hexaploid wheat cultivars, showed higher TWT compared to the mapping population and all durum wheat check cultivars at Kernen 2010 (Table 12). Averaged over years, the checks and the mapping population did not reveal any significant differences in TWT (Table 12).

Table 12: Least Square (LS) Means of heading date (HD), plant height (PH), yield (YLD), thousand-kernel weight (TKW) and test weight (TWT) in two environments (Kernen 2009 and Kernen 2010), using a randomized complete block design with two replications.

Accessions	HD (day)			PH (cm)			YLD (kg/ha)			TKW (g)			TWT (kg/hl)		
	KN09	KN10	Mean	KN09	KN10	Mean	KN09	KN10	Mean	KN09	KN10	Mean	KN09	KN10	Mean
Goodeve	56.0 e	57.0 cd	56.5 fg	88.5 c	98.5 abc	93.5 abc	3210 d	3792 abc	3501 cdef	36.2 f	43.7 d	40.0 e	80.5 f	78.4 a	79.4 abc
Waskada	56.0 e	55.5 e	55.8 g	96.0 ab	101 ab	98.5 ab	4796 abc	3087 cde	3941 bcde	37.3 f	38.6 e	37.9 e	82.6 cd	79.5 a	81.0 a
AC Avonlea	58.5 d	56.5 de	57.5 ef	92.5 abc	81.5 def	87.0 cd	3699 cd	1817 f	2758 f	45.4 cde	44.5 cd	45.0 cd	82.0 de	75.5 b	78.8 abcd
AC Morse	58.5 d	58.0 bc	58.3 de	87.5 c	91.5 abcde	89.5 c	4669 abc	3441 bcd	4055 abcd	48.2 abc	46.4 bcd	47.3 bcd	81.5 ef	73.9 cd	77.7 bcd
AC Navigator	62.0 a	56.5 de	59.3 bcd	78.5 d	80. 5 ef	79.5 de	4468 abcd	2431 ef	3449 cdef	49.3 ab	48.7 b	49.0 abc	83.6 ab	70.7 e	77.1 cd
Brigade	62.5 a	60.0 a	61.3 a	98.5 a	102.5 a	100.5 a	4833 abc	3498 bcd	4165 abc	51.2 a	49.7 b	50.4 ab	81.4 ef	73.2 d	77.3 bcd
Commander	61.5 ab	58.0 bc	59.8 bc	71.0 e	76.5 f	73.8 e	3276 d	2521 ef	2899 ef	51.4 a	53.6 a	52.5 a	83.1 bc	70.9 e	77.0 d
Eurostar	61.0 abc	59.0 ab	60.0 ab	92.5 abc	95.0 abc	93.8 abc	3322 d	2655 def	2988 def	46.9 bcd	49.1 b	48.0 bc	83.9 ab	73.9 cd	79.0 abcd
Strongfield	59.0 d	58.5 b	58.8 bcde	92.0 abc	86.0 cdef	89.0 c	5261 ab	2200 f	3730 bcdef	44.2 de	46.2 bcd	45.2 cd	83.0 bc	74.4 bcd	78.7 abcd
CDC Verona	60.0 cd	59.0 ab	59.5 bcd	91.5 abc	96.5 abc	94.0 abc	4394 abcd	3120 cde	3757 bcdef	47.3 bcd	49.0 b	48.2 bc	84.1 a	75.3 b	79.7 ab
DT780	59.5 bcd	59.0 ab	59.3 bcd	90.0 bc	94.0 abcd	92.0 abc	5288 ab	4202 ab	4745 ab	42.7 e	48.1 bc	45.4 cd	82.3 cde	74.5 bc	78.4 bcd
Sm1 "-" (S)	59.4 d	58.7 b	59.1 bcd	91.3 bc	93.0 bc	92.1 c	4920 b	3043 de	3977 bc	47.0 bc	47.4 b	47.2 c	82.8 c	74.1 c	78.4 bcd
Sm1 "+" (R)	59.3 d	58.5 b	58.9 cd	92.1 bc	93.9 abc	93.0 bc	5310 a	4227 a	4769 a	45.1 de	44.5 d	44.8 d	82.2 d	74.2 c	78.2 bcd
LSD $_{0.05}$ ***(Sm1"-" vs Sm1"+")***	0.269	0.201	0.193	1.09	1.85	1.15	188	128	237	0.525	0.519	0.459	0.147	0.186	1.02

For each column, means followed by the same letter are not significantly different (p= 0.05).

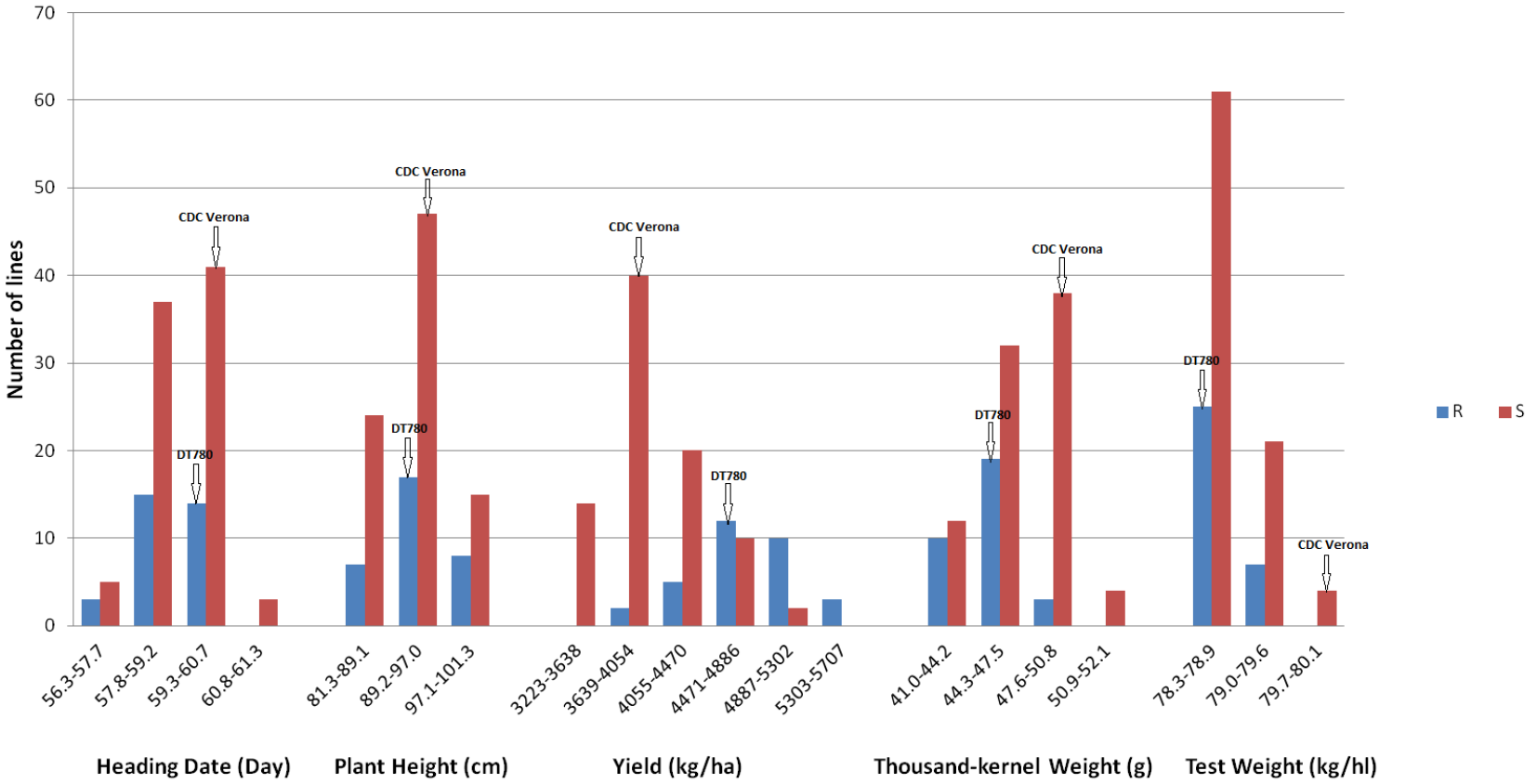

Figure 6: Frequency distribution of midge resistant lines (R) and susceptible lines (S) of the RIL mapping population derived from CDC Verona/DT780 for heading date (HD), plant height (PH), yield (YLD), thousand-kernel weight (TKW) and test weight (TWT) based on the LS means from two environments (Kernen 2009 and Kernen 2010).

Table 13: Monthly growing season precipitation (mm) received at the Kernen Crop Science Research Farm in 2010. The 30-year average is presented for comparison.

Year	May	June	July	August	Sept	Total	Average
2010	120	150	91	58	100	519	103.8
30-year average	42	71	61	38	30	242	48.4

4.4.2 Yield (YLD)

Yield was variable between RILs of the mapping population in 2009 and 2010 (Appendix 2). The YLD ranged from 3520 to 6593 kg ha^{-1} in the mapping population in 2009, and from1774 to 5307 kg ha^{-1} in 2010 (Appendix 2). Frequency distribution showed that lines carrying *Sm1* mainly expressed higher YLD than lines lacking *Sm1* (Figure 6). Transgressive segregation was evident for YLD, and most lines carrying *Sm1* within RIL lines showed greater YLD than 'CDC Verona', in the combined analysis (Appendix 2 and Figure 6). 'DT780' had the highest yield among check cultivars over both environments (Table 12). 'AC Avonlea' consistently showed lower YLD than other durum and spring wheat checks in two environments (Table 12). In both environments, lines carrying *Sm1* in the mapping population were significantly higher yielding than those lacking *Sm1* (Table 12). At Kernen 2010, where OWBM pressure was high, *Sm1* carriers out-yielded those lacking *Sm1* by 39%. At Kernen 2009, this difference was only 8% (Table 12). In addition to high OWBM pressure in 2010, average rainfall during the growing season was 114% higher than the 30-year average (Table 13). Averaged over both years of testing, the lines carrying *Sm1* in the mapping population yielded 20% more than the lines not carrying *Sm1* (Table 12). 'Goodeve', a CWRS variety that carries *Sm1* was significantly lower yielding than the CWRS cultivar 'Waskeda' in 2009, but not significantly different in 2010 (Table 12). 'Brigade' revealed relatively higher yields compared to other durum wheat check cultivars in both environments, but lines from the mapping population that carried *Sm1* showed higher yields than 'Brigade' at Kernen 2010 (Table 12).

4.4.3 Thousand-kernel Weight (TKW)

The durum wheat check cultivars 'AC Avonlea', 'AC Morse', 'AC Navigator', 'Brigade', 'Commander', 'Eurostar', 'Strongfield' and 'CDC Verona' had higher TKW than the CWRS checks 'Waskada' and 'Goodeve', in all environments (Table 12). 'CDC Verona' revealed higher TKW than that of 'DT780', from individual environments and combined analysis, (Table 12 and Figure 6) but was only significantly higher at Kernen 2009 (Table 12). 'Commander' and 'Brigade' showed the highest TKW among durum wheat check cultivars and significantly higher than *Sm1* carriers at both Kernen 2009 and Kernen 2010 (Table 12). The CWRS checks 'Waskada' and 'Goodeve' showed lower TKW than the mapping population (Table 12). On average, the *Sm1* carriers had relatively lower TKW than all the durum wheat checks in both environments (Table 12). At Kernen in 2010, the *Sm1* carriers also had significant lower TKW than that of 'DT780' at Kernen 2010 ($P<0.05$) (Table 12). At Kernen 2009, TKW of *Sm1* carriers were higher than that of 'DT780', but lower than that of 'CDC Verona' (Table 12). On average, *Sm1* carriers had statistically lower TKW compared to non-carriers (Table 12). However, some *Sm1* carriers had higher TKW than *Sm1* non-carriers (Appendix 2). This data suggests that it is possible to select lines carrying *Sm1* and expressing higher TKW (Appendix 2 and Table 12).

4.4.4 Falling Number (FN)

At Indian Head 2009 and Kernen 2009, the range in FN was from 318 to 471 sec and 260 to 412 sec, respectively, in the RIL mapping population (Appendix 2). The average FN was also higher in *Sm1* carriers of the mapping population, compared to those lacking *Sm1*, but this difference was only significantly different at Kernen 2009 (Table 14). In contrast, the range in FN at Kernen 2010 was from 61 to 164 sec in the RIL population (Appendix 7), well below that observed in 2009. This was likely due to excessive rainfall during the September (Table 13), which would have resulted in increased potential for pre-harvest sprouting. In 2010, lower average FN was

observed in *Sm1* carriers (Table 14), particularly compared to 'CDC Verona' and 'AC Morse' which expressed higher FN than most other durum wheat check cultivars (Table 14). The CWRS cultivar 'Waskada' consistently had a high FN across the three environments (Table 14), and in particular, it exhibited the highest FN at Kernen 2010. 'Waskada' is known to express good tolerance to pre-harvest sprouting (Fox et al. 2009).

4.4.5 Grain Protein Content (GPC)

At all three environments, significant transgressive segregation of GPC was observed in the RIL population (Appendix 2). On average, the *Sm1* carriers had lower GPC than 'DT780', which expressed significantly lower GPC than 'CDC Verona' at Kernen 2010 (Table 14). However, when averaged over environments, 'CDC Verona' and 'DT780' did not differ significantly (Table 14 and Figure 7). RIL lines showed greater and lower expression of GPC than either of 'DT780' and 'CDC Verona' (Appendix 2 and Figure 7). Most lines carrying *Sm1* had lower GPC, compared with *Sm1* non-carriers, when averaged over environments (Appendix 2 and Figure 7).

GPC at Kernen 2010 was significantly higher than that in the other two environments (Table 14). At Indian Head 2009 and Kernen 2010, the *Sm1* carriers had a significantly lower GPC than *Sm1* non-carriers ($P<0.05$), but no significant difference was detected in 2009 at Kernen (Table 14). Meanwhile, on average, the *Sm1* carriers had a lower GPC than 'Strongfield', a cultivar that expresses high GPC, but no significant difference was detected when over environments (Table 14). However, some lines carrying *Sm1* expressed higher GPC as observed from the ranges of GPC in two test environments (Appendix 3 and Table 14). A 4.3% difference in GPC was observed at Indian Head 2009; and a 6.2% difference was observed at Kernen 2010 (Table 14). At both Indian Head and Kernen 2010, on average, the *Sm1* carriers expressed similar GPC to 'Commander' and 'AC Navigator' (Table 14). 'Commander' (Clarke et al. 2006) and 'AC Navigator' are known to express low GPC relative to 'Strongfield' (Clarke et al. 2001).

Table 14: Least Square (LS) Means of falling number (FN), grain protein content (GPC) and yellow pigment (YP) (14% moisture basis) in three environments (Indian Head 2009, Kernen 2009 and Kernen 2010) using a randomized complete block design with two replications.

Accessions	FN (sec)				GPC (%)				YP (mg/kg)			
	IH09	**KN09**	**KN10**	**Mean**	**IH09**	**KN09**	**KN10**	**Mean**	**IH09**	**KN09**	**KN10**	**Mean**
Goodeve	431	390	114	312	13.2	14.5	19.4	15.7	2.40	3.15	3.45	3.00
	abc	a	bc	bc	ab	abcd	ab	ab	g	g	g	e
Waskada	391	381	317	363	13.8	15.6	19.4	16.2	0.645	0.95	2.40	1.33
	cd	ab	a	a	a	ab	ab	a	h	h	h	f
AC Avonlea	393	353	95.0	280	12.7	14.9	19.4	15.7	5.79	6.70	8.20	6.90
	cd	abcd	cde	bcde	abcd	abc	ab	ab	e	ef	def	d
AC Morse	462	364	133	320	11.8	14.6	18.6	15.0	5.25	6.49	7.65	6.47
	ab	abc	b	b	bcdef	abcd	bcd	bcd	f	f	f	d
AC Navigator	475	352	73.0	300	11.8	13.7	18.1	14.6	6.25	7.25	9.15	7.55
	a	abcd	ef	bcd	bcdef	bcd	de	bcd	d	cde	a	ab
Brigade	357	322	62.0	247	10.8	15.9	18.0	14.9	6.40	7.65	8.45	7.50
	d	d	f	e	f	a	de	bcd	bcd	abc	bcde	abc
Commander	460	360	62.0	294	12.0	12.9	18.6	14.5	6.50	8.00	8.90	7.80
	ab	abcd	f	bcd	bcdef	d	bcd	cd	bcd	a	ab	a
Eurostar	433	366	85.0	295	11.1	12.8	18.8	14.2	6.15	6.65	8.10	6.97
	abc	abc	def	bcd	efg	d	abcd	d	de	f	ef	cd
Strongfield	414	357	71.5	281	12.5	14.8	19.6	15.6	6.65	7.40	8.65	7.57
	bc	abcd	ef	bcde	abcde	abcd	a	abc	abc	bcd	abde	ab
CDC Verona	413	359	103	292	11.3	13.5	19.3	14.7	6.30	7.60	8.70	7.53
	bc	abcd	cd	bcd	cdef	cd	abc	bcd	cd	abcd	abd	ab
DT780	403	353	62.5	273	11.7	13.9	18.4	14.7	6.75	7.70	8.45	7.63
	cd	abcd	f	cde	bcdef	abcd	cde	bcd	ab	abc	bcde	ab
Sm1 "-" (S)	402	339	85.7	275	12.1	14.5	18.9	15.2	6.33	7.22	8.63	7.39
	c	cd	de	d	ce	abc	abc	bc	d	d	b	b
Sm1 "+" (R)	408	354	70.3	277	11.6	14.5	17.8	14.6	6.77	7.67	8.80	7.75
	c	b	f	d	dfg	abc	e	d	a	ab	ac	a
LSD $_{0.05}$ ***(Sm1"-" vs Sm1"+")***	7.53	5.91	3.65	25.8	0.230	0.293	0.136	0.521	0.05	0.081	0.086	0.177

For each column, means followed by the same letter are not significantly different (p= 0.05).

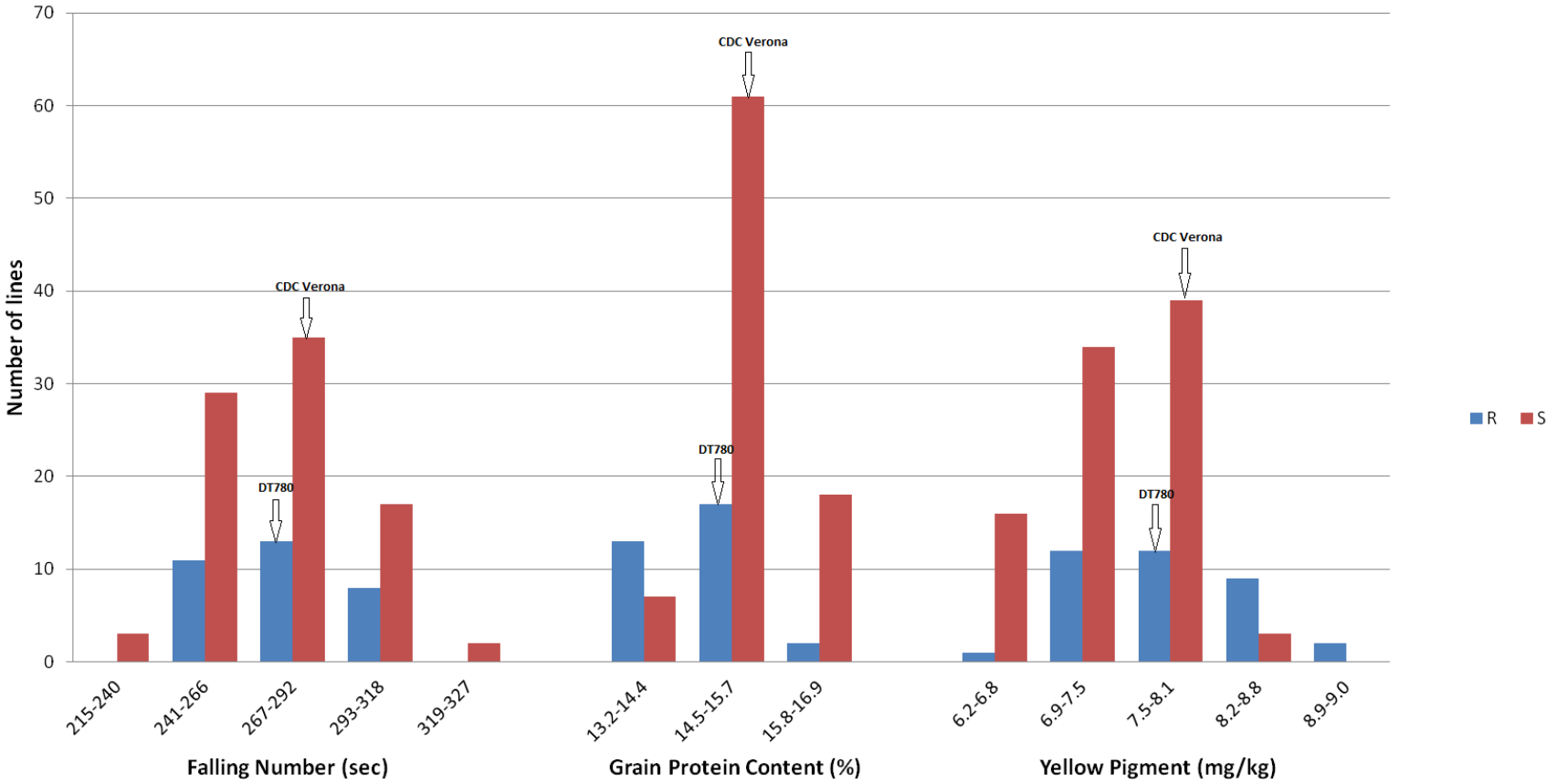

Figure 7: Frequency distribution of midge resistant lines (R) and susceptible lines (S) of the RIL mapping population derived from CDC Verona/DT780 for falling number (FN), grain protein content (GPC) and yellow pigment (YP) based on the LS means across three environments (Indian Head 2009, Kernen 2009 and Kernen 2010).

4.4.6 Yellow Pigment (YP)

Like GPC, significant transgressive segregation of YP was observed in the RIL mapping population (Appendix 3 and Figure 7). Averaged over all three environments, the range in YP was from 6.2 to 9.0 mg/kg (Appendix 3 and Figure 7). More lines carrying *Sm1* expressed significant higher YP than lines lacking *Sm1* (Figure 7). The average YP content of *Sm1* carriers was higher than that in *Sm1* non-carriers of the mapping population across environments, despite 'DT780' and 'CDC Verona' showing similar expression (Table 14). On average, the *Sm1* carriers had higher YP values than the parents, but the difference was observed only in two environments (Indian Head 2009 and Kernen 2010) (Table 14). Averaged over all three environments, the mean YP of lines carrying *Sm1* was similar to 'Commander' and 'AC Navigator', which were known to express high levels of YP (Clarke et al. 2006; 2001) (Table 14). As expected, the CWRS cultivars 'Goodeve' and 'Waskada' expressed low YP when compared to all durum wheat cultivars (Table 14). Of the durum wheat check cultivars, 'AC Morse' expressed the lowest YP, averaged three environments (Table 14).

4.4.7 Gluten Strength (GI, WETG, ST and RELAX)

Averaged over three test environments, the GI ranged from 11.2 to 77.6 % in the RIL population (Appendix 3). 'DT780' had a lower GI than 'CDC Verona' in all three environments, but the difference between the two parents was significant only at Indian Head 2009 (Table 15). Averaged over all three environments, 'Commander' and 'Brigade' were not significantly different in GI (Table 15). When averaged over all environments, there was significant transgressive segregation in the RIL population, with some lines expressing lower GI than 'DT780' and several lines expressing GI greater than 'CDC Verona' (Appendix 3 and Figure 8).

On average, the lines carrying *Sm1* in the mapping population expressed reduced gluten strength properties, and the differences in GI were consistent across all three environments (Table 15). The GI was statistically lower in *Sm1* carriers compared with 'CDC Verona' and 'DT780', across three environments (Table 15). In contrast, the

average GI of the non-carriers was statistically similar to 'CDC Verona' and 'DT780' (Table 15), but a higher frequency lines lacking *Sm1* had a high GI (Figure 8). Although the *Sm1* carriers had a lower GI than *Sm1* non-carriers, several carriers expressed GI similar to 'Strongfield' and 'AC Morse' (Table 15 and Appendix 3). However, none of the RIL's was as strong (high GI) as 'Commander' (Figure 8 and Appendix 3). Most lines in the RIL population expressed higher GI than 'AC Avonlea', across environments as well as in individual environments (Appendix 3). 'AC Avonlea' is a known weak gluten durum wheat cultivar, and was found to have the lowest GI when compared to other durum check cultivars (Table 15). 'DT780', had intermediate GI that was statistically similar to durum wheat cultivars 'AC Morse' and 'Strongfield' in all environments (Table 15).

Lower average WETG were observed in *Sm1* carriers, but not all lines carrying *Sm1* revealed reduced WETG, from Indian Head 2009, Kernen 2010 and over all environments (Table 15, Figure 8 and Appendix 3). However, on average, WETG of *Sm1* carriers were statistically higher at Kernen 2009 (Table 15). In addition, statistically shorter average ST were also observed in *Sm1* carriers, but some of the *Sm1* carriers showed higher values, compared to non-carriers, in all three environments (Table 15 and Appendix 3). Overall, durum wheat cultivars 'Brigade' and 'Commander' both presented the longest ST among checks (Table 15). On average, lines carrying *Sm1* had longer relaxation than *Sm1* lacking lines in all three environments (Table 15). Gluten relaxation was significantly different only at Indian Head 2009, but these differences were small (Table 15).

Table 15: Least Square (LS) Means of gluten index (GI), wet gluten content (WETG), stretching time (ST) and relaxation (RELAX) (14% moisture basis) in three environments (Indian Head 2009, Kernen 2009 and Kernen 2010) using a randomized complete block design with two Replications.

Accessions	GI (%)				WETG (%)				ST (sec)				RELAX (BU)			
	IH09	**KN09**	**KN10**	**Mean**	**IH09**	**KN09**	**KN10**	**Mean**	**IH09**	**KN09**	**KN10**	**Mean**	**IH09**	**KN09**	**KN10**	**Mean**
Goodeve	61.2	52.2	49.1	54.1	32.3	36.7	53.7	40.9	15.5	9.00	6.00	10.2	221	257	266	248
	de	cdef	bc	cde	a	abc	a	ab	cde	b	cd	cd	fh	ab	a	abcd
Waskada	62.5	55.2	44.6	54.1	33.4	41.0	51.9	42.1	11.0	22.5	6.00	13.2	258	219	263	246
	cde	bcdef	cde	cde	a	a	a	a	cde	ab	cd	bcd	abcd	bc	ab	abcd
AC Avonlea	25.8	40.1	32.0	32.6	27.7	36.1	45.7	36.5	6.00	7.00	6.00	6.33	288	234	255	259
	g	ef	fg	f	a	abc	bc	abc	de	b	cd	cd	a	abc	abc	ab
AC Morse	46.1	37.9	48.0	44.0	24.5	33.8	43.4	33.9	7.50	6.00	9.00	7.50	271	276	249	265
	ef	ef	bcd	def	a	abcde	cd	abc	de	b	bcd	cd	ab	a	abcd	a
AC Navigator	80.0	74.7	44.2	66.3	22.5	28.4	41.7	30.9	28.0	15.0	9.00	17.3	202	229	230	220
	abc	abc	cde	abc	a	defg	def	c	bc	b	bcd	bcd	hi	abc	defg	def
Brigade	96.1	69.7	64.3	76.7	17.6	34.2	39.6	30.4	80.5	21.5	16.5	39.5	178	210	223	203
	a	abcd	a	a	a	abcde	f	c	a	ab	a	a	i	bc	efg	fg
Commander	96.9	85.9	63.9	82.2	22.4	27.8	42.5	30.9	76.5	46.5	18.0	47.0	175	184	215	191
	a	a	b	a	a	eg	de	c	a	a	a	a	i	c	g	g
Eurostar	89.6	77.8	53.0	73.4	19.7	24.0	44.4	29.3	35.5	23.0	15.5	24.7	197	221	222	213
	ab	ab	b	ab	a	g	cd	c	b	ab	ab	b	hi	bc	fg	efg
Strongfield	49.6	46.6	46.5	47.6	26.7	33.2	45.7	35.1	9.50	8.00	9.50	9.00	252	246	240	246
	ef	def	bcde	def	a	bcde	bc	abc	de	b	bcd	cd	bcdef	ab	cdef	abcd
CDC Verona	74.6	58.0	45.8	59.5	20.3	31.0	48.3	33.2	18.0	14.5	7.00	13.2	225	236	256	239
	bcd	bcde	bcde	bcd	a	bcdeg	b	bc	bcde	b	cd	bcd	efgh	abc	abc	abcde
DT780	48.3	42.3	39.3	43.3	24.6	31.5	43.1	33.0	7.50	6.00	6.00	6.50	261	259	245	255
	ef	ef	ef	ef	a	bcde	cde	bc	de	b	cd	cd	abcd	ab	bcd	abc
Sm1 "-" (S)	55.7	50.2	42.3	49.4	25.2	31.6	43.6	33.5	14.4	14.9	10.4	13.2	240	239	236	238
	e	e	de	de	a	cef	d	c	d	b	c	c	dfg	ab	def	cd
Sm1 "+" (R)	45.5	40.7	28.9	38.4	23.4	33.5	41.0	32.7	9.77	10.6	6.22	8.87	247	245	237	243
	f	f	g	f	a	bd	ef	c	e	b	d	d	ce	ab	de	b
LSD $_{0.05}$ ***(Sm1"-" vs Sm1"+")***	2.65	3.26	1.22	2.14	3.66	1.08	0.436	1.88	1.38	4.46	0.978	1.68	4.34	8.14	3.02	3.67

For each column, means followed by the same letter are not significant different (p= 0.05).

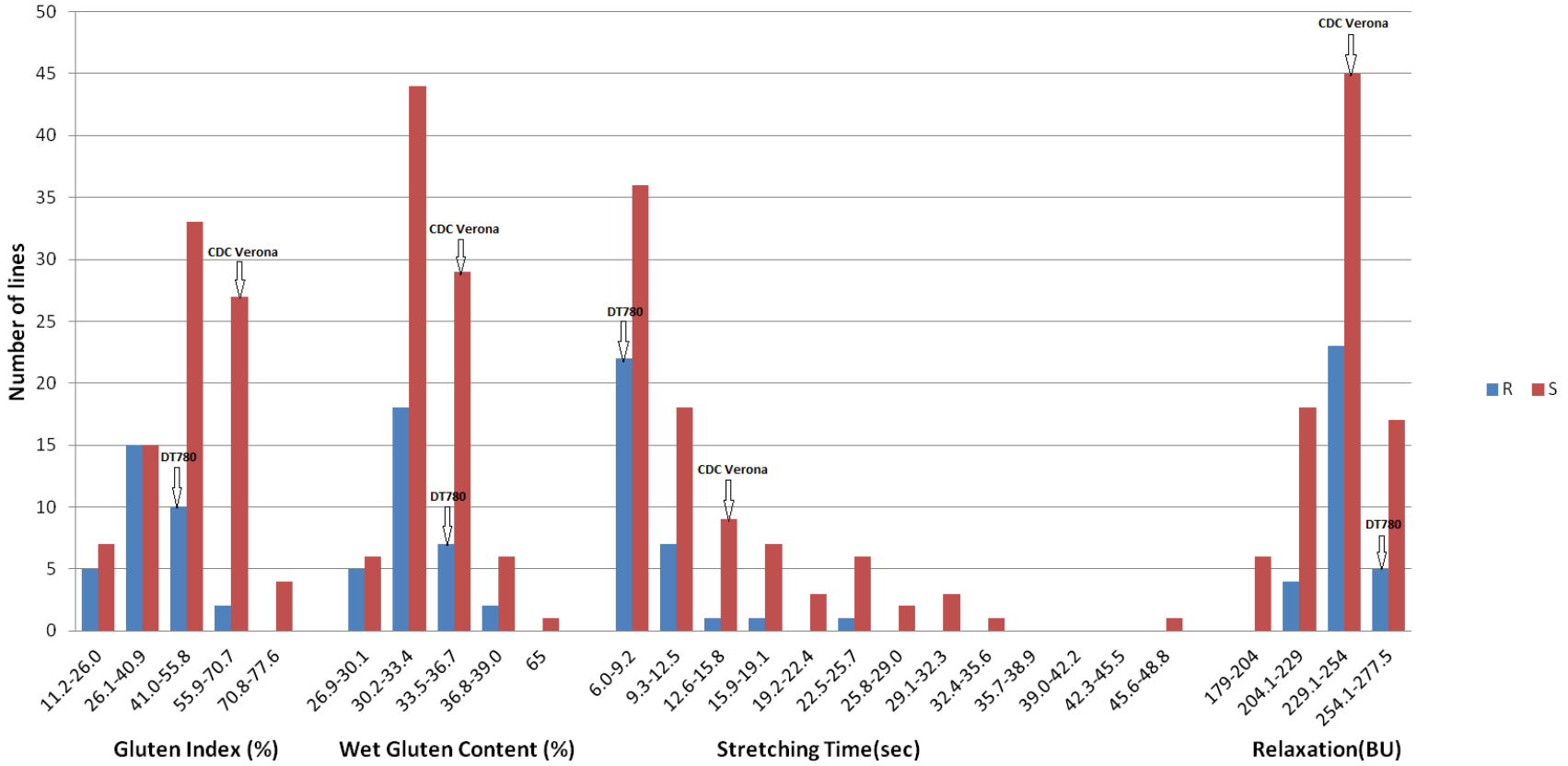

Figure 8: Frequency distribution of midge resistant lines (R) and susceptible lines (S) of the RIL mapping population derived from CDC Verona/DT780 for gluten index (GI), wet gluten content (WETG), stretching time (ST) and relaxation (RELAX) based on the LS means across three environments (Indian Head 2009, Kernen 2009 and Kernen 2010).

4.5 QTL Mapping of *Sm1* in Durum Wheat

4.5.1 Genetic Mapping of Sm1

In hexaploid wheat, *Sm1* has been shown to be inherited as a single gene that has been mapped to the short arm of chromosome 2B. To determine the size of the *Sm1* introgression into durum wheat from hexaploid wheat, a genetic map of *Sm1* was constructed using the CDC Verona/DT780 RIL mapping population. In total, 14 markers (ESTs, SSRs and DArTs) were polymorphic in the mapping population and localized to chromosome 2B. Those markers were assembled into one linkage group and were all assigned to chromosome 2B and spanned approximately 58 cM. The total size of the *Sm1* introgression was approximately 11cM (Figure 10, 11, 12 and 13).

According to the physical location of *Sm1*, 31 EST markers were designed and evaluated from the sequences of 8 wheat ESTs previously localized to BIN 2BS-4. Nine of these markers produced amplicons that were polymorphic between CDC Verona and DT780 but only one was mapped in the RIL population (Figure 9). Primers (BE443737_316) designed from the sequences of BE443737 produced two polymorphic fragments (upper arrows for BE44373_316.1; lower arrows for BE443737_316.2) (Figure 9) and mapped 26 cM proximal to *Sm1*.

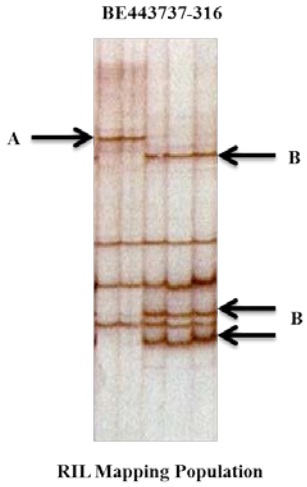

Figure 9: Polymorphisms was detected at BE443737-316 in the RIL mapping population (CDC Verona/DT780) using SSCP. Arrows indicate those polymorphic fragments that scored into A and B (A: CDC Verona allele; B: DT780 allele)

4.5.2 Segregation Distortion Analysis

Field observations enabled identification of resistant or susceptible lines in the mapping population (Appendix 1, 2 and 3). Seeds of F_5-derived lines were advanced by four generations of selfing and harvesting in bulk to obtain the $F_{5:9}$ mapping population. The genetic segregation ratio of the RIL population was expected to be 1R: 1S based on segregation of a single Mendelain factor (1 gene) (Table 16), as others have shown it to be a single gene (Lamb et al. 2000; Thomas et al. 2005). However, the observed data did not fit a 1:1 ratio (Chi-square test, P <0.001), but fit a 3:1 ratio (Table 16), suggesting segregation of a two independently segregating genes.

Table 16: Segregation ratios of SSR, EST, DArT markers in the mapping population (CDC Verona/ DT780 RIL population).

Marker loci	Linkage group	Segregation ratio			
		Frequency of parental alleles (%)		χ2 P value	
		CDC Verona allele	DT780 allele	1:1 (Expected)	3:1 (Observed)
BE443737-316.1	2B	42	58	ns	P<0.001
BE443737-316.2	2B	41	59	ns	P<0.001
Xwmc382.2	2B	50	50	ns	P<0.001
2B_wPt-0100	2B	52	48	ns	P<0.001
2B_wPt-1634	2B	53	47	ns	P<0.001
2B_wPt-1842	2B	56	44	ns	P<0.001
2B_wPt-2106	2B	57	43	ns	P<0.001
Sm1	2B	73	27	P<0.001	ns
Xwmc489	2B	73	27	P<0.001	ns
Xgwm210.1	2B	73	27	P<0.001	ns
Xgwm210.2	2B	75	25	P<0.001	ns
Xwmc382.1	2B	80	20	P<0.001	ns
barc124	2B	80	20	P<0.001	ns
Xgwm614	2B	80	20	P<0.001	ns
Xgwm429	2B	86	14	P<0.001	p<0.01
Xgwm410	2B	84	16	P<0.001	p<0.05
Xgwm148	2B	84	16	P<0.001	p<0.05
Xwmc332	2B	73	27	P<0.001	ns

*p<0.05; **p<0.01; ***p<0.001; ns: not significant

From Table 16, chi-square test represents the probability of deviation from the segregation ratios 1S: 1R and 3S: 1R. Seven loci of linked markers had P-value greater than 0.05 indicate that the observed ratios were not significantly different from expected ratio (1S: 1R) (Table 16). Ten polymorphic loci out of 17 loci (59 %)

significantly segregate away from the expected Mendelian inheritance ratio of 1:1 (Table 16). Likewise, eight loci from the genetic map had no significant difference from segregation ratio 3:1 (Table 16). Meanwhile, three polymorphic loci showed segregation distortion fit a 3:1 ratio (CDC Verona allele: DT780 allele), suggesting that these three loci (two markers) are closely linked with *Sm1* (Table 16). Taken together, these confirm that *Sm1* is a single gene, but the presence of segregation distortion skewed the population to the susceptible parent alleles.

4.6 Quantitative Trait Loci (QTL) Analysis

Significant QTLs associated with most measured traits were detected in the CDC Verona/DT780 mapping population, based on least squares means in individual environments as well as across all environments (Figure 10, 11, 12 and 13). Significant QTLs associated with midge-damaged seeds were detected at Kernen 2010 (Figure 10). Significant QTLs associated with YP, GI, WETG, YLD, TWT and TKW were detected at Kernen 2009 (Figure 11, 12 and 13). At Kernen 2010, most traits had significant QTLs with the exceptions of HD, PH, YP and RELAX (Figure 11, 12 and 13).

Three significant QTLs for damaged seed (categories 1, 2 and 4) were identified and all were flanked by *Sm1* introgression at Kernen 2010 (Figure 10). A significant QTL for TWT was identified and flanked by *Sm1* and *Xwmc 489* at Kernen 2009 (Figure 11). Meanwhile, a small QTL for TWT was also detected near *Xwmc* 332, at Kernen 2010 (Figure 11). Strong QTLs associated with TKW were detected by the closest markers *Sm1* and *Xwmc 489,* at Kernen 2009, Kernen 2010 as well as in the combined analysis across both environments (Figure 11). Two small QTLs for TKW were also detected in another region beyond the *Sm1* introgression (Figure 11). QTLs for yield were found in Kernen 2009, Kernen 2010 and over the two environments (Figure 11). Extremely strong QTLs (LOD=19.2) associated with yield were detected at Kernen 2010 (Table 17 and Figure 11). In contrast, a small QTL (LOD=2.3) for yield was detected in Kernen 2009 (Table 17 and Figure 11).

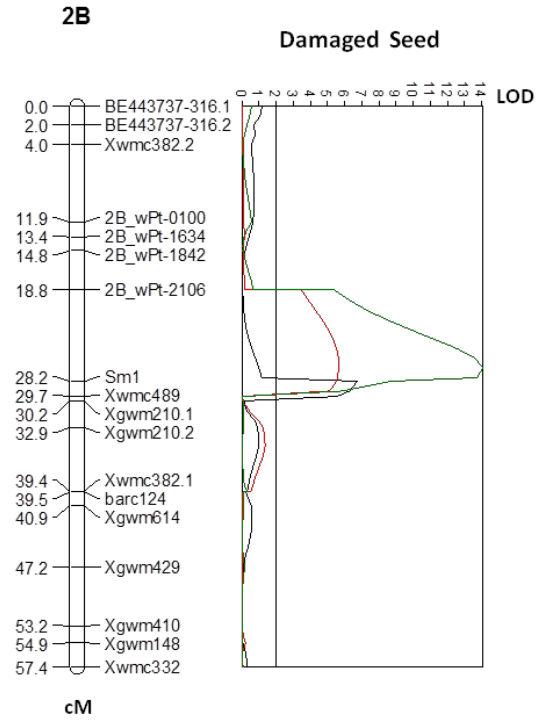

Figure 10: Genetic linkage map of the *Sm1* region on chromosome 2B and the positions of QTLs for midge damaged seeds. Significance is declared for QTL to the right of the vertical line located at LOD 2. **Black line:** Category 1**; Red line:** Category 2**; Green line:** Category 4**.**

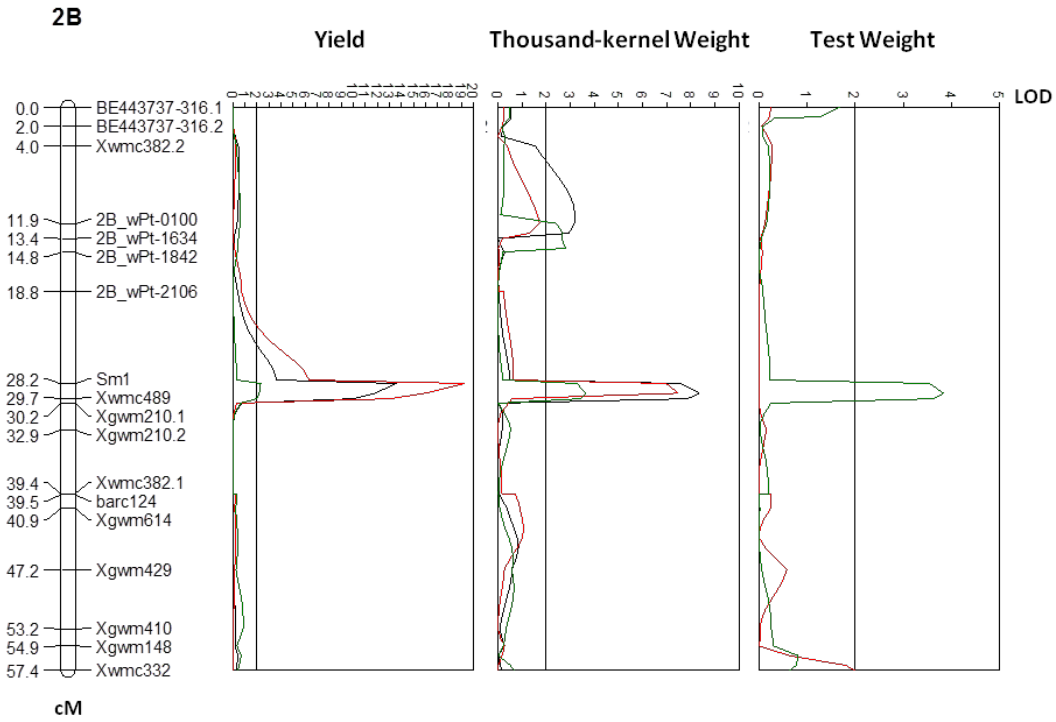

Figure 11: Genetic linkage map of the *Sm1* region on chromosome 2B and the positions of QTLs for yield (YLD), thousand-kernel weight (TKW) and test weight (TWT). Significance is declared for QTL to the right of the vertical line located at LOD 2. **Black line:** Combined analysis; **Red line:** Kernen 2010; **Green line:** Kernen 2009.

QTLs associated with YP were detected with similar LOD scores (approx. 3), flanked by *Sm1* and *Xgwm210*, at Indian Head 2009, Kernen 2009 and in the combined analysis (Figure 12). A significant QTL associated with FN was only detected at Kernen 2010, which is flanked by *Sm1* introgression (Figure 12). GPC had significant QTLs, overlapping with the region of *Sm1* introgression at Kernen 2010; nevertheless, two relatively small QTLs for GPC were flanked by *Sm1* and *Xwmc489*, at Indian Head 2009 and in the combined analysis (Figure 13). Analysis of WETG revealed that significant strong QTLs overlap the *Sm1* introgression at Kernen 2010 (Figure 13). QTLs associated with both ST and GI were detected in the same region and flanked by *Sm1* and *Xgwm210*, at Kernen 2010 and in the combined analysis (Figure 13). Furthermore, another QTL associated with ST was found located upstream of the primary QTL, and may influence on the main QTL (Figure 13).

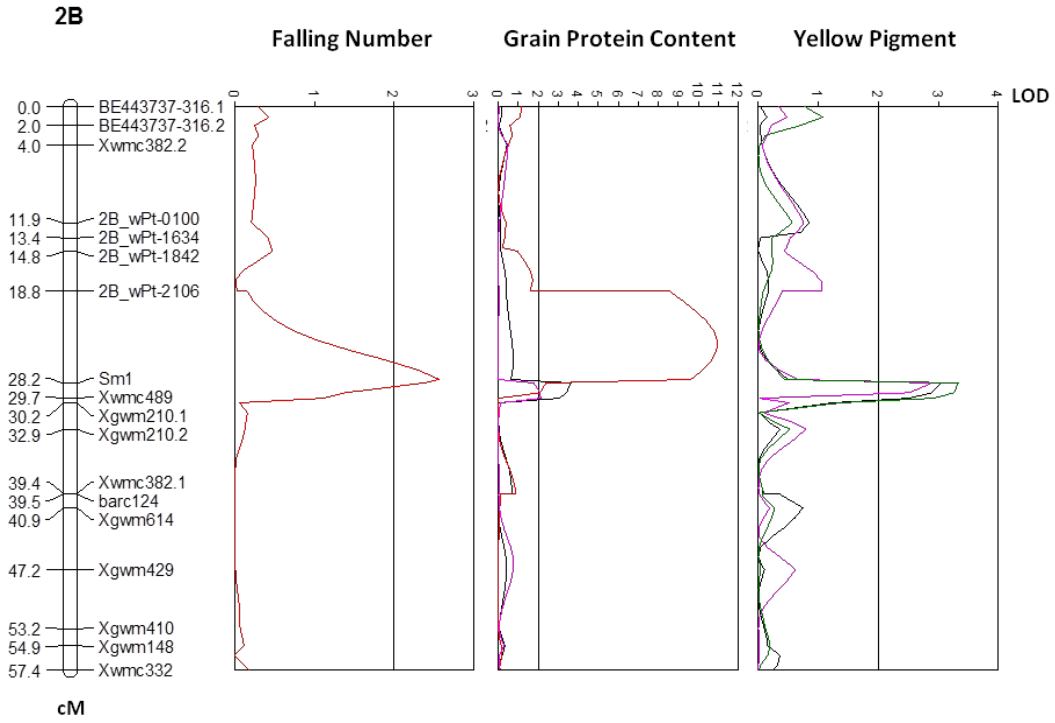

Figure 12: Genetic linkage map of the *Sm1* region on chromosome 2B and the positions of QTLs for falling number (FN), grain protein content (GPC) and yellow pigment (YP). Significance is declared for QTL to the right of the vertical line located at LOD 2. **Black line:** Combined analysis; **Red line:** Kernen 2010; **Green line:** Kernen 2009; **Purplish red line:** Indian Head 2009.

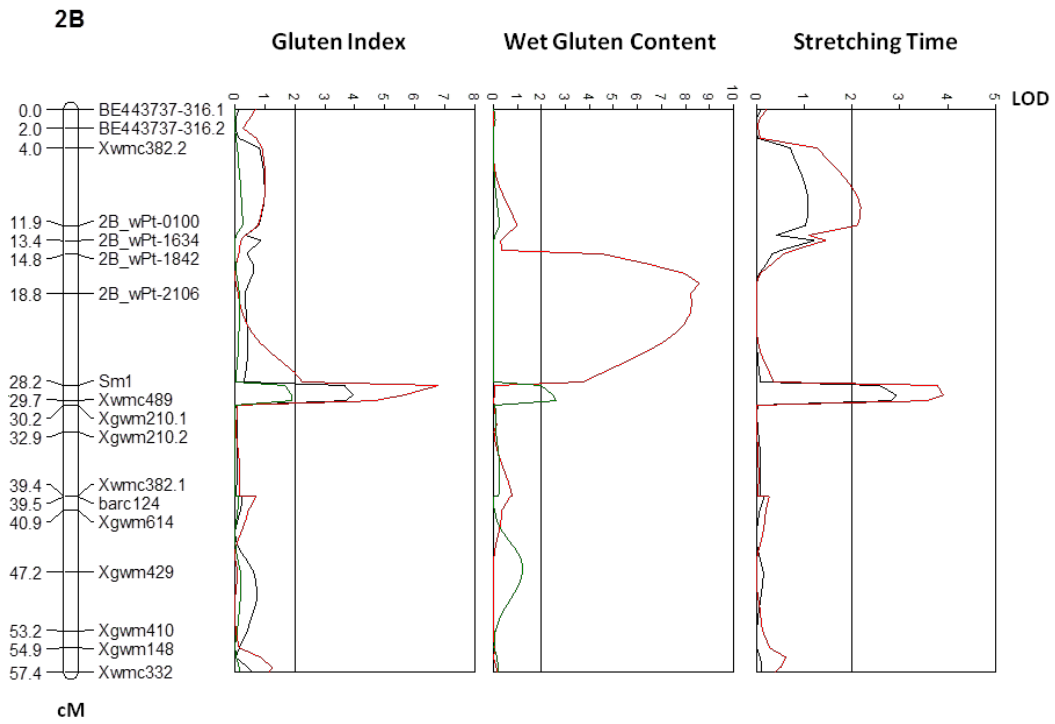

Figure 13: Genetic linkage map of the *Sm1* region on chromosome 2B and the positions of QTLs for gluten index (GI), wet gluten content (WETG) and stretching time (ST). Significance is declared for QTL to the right of the vertical line located at LOD 2. **Black line:** Combined analysis; **Red line:** Kernen 2010; **Green line:** Kernen 2009; **Purplish red line:** Indian Head 2009.

Overall, most QTLs were detected at Kernen 2010 and Kernen 2009. TKW, YLD, GI, ST and three categories (1, 2 and 4) of damage seed all revealed strong significant QTLs at Kernen 2010 (Figure 10, 11, 12 and 13). Strong QTLs were also similarly detected in the combined analysis (Figure 11, 12 and 13). QTLs for TWT, TKW, YLD, YP, WETG and GI (LOD=1.88), were small but meaningful at Kernen 2009 (Table 17; Figure 11, 12 and 13).

'CDC Verona' contributed the alleles for elevated TKW in the mapping population (Table 17). *Sm1* explained 11.0% to 25.3% of the variation in TKW (Table 12). Elevated FN values were also contributed by 'CDC Verona' alleles, only 8.8% of the variation was explained by *Sm1*, at Kernen 2010 (Table 17). 'CDC Verona' also contributed the alleles for elevated GPC, GI and ST, in different environments. In terms of GPC, *Sm1* explained 6.4% to 13.1% of the variation (Table 17). For GI, *Sm1* explained 6.3% to 13.2% of the variation (Table 17). For ST, *Sm1* explained 9.5% to 13.5% of the variation (Table 17).

'DT780' contributed alleles for elevated YLD at Kernen 2009 and Kernen 2010 and in the combined analysis (Table 17). The variation of YLD explained by *Sm1* ranged from 8.5% to 52.5 % (Table 17). Meanwhile, 'DT780' also contributed alleles for elevated YP, with the variation explained by *Sm1* ranging from 10.6% to 12.1% (Table 17). TWT and WETG did not consistently reveal effects of *Sm1*; however, QTL×Environment interactions were significant for these traits (Table 17).

Table 17: QTLs of associated traits detected in the CDC Verona/DT780 mapping population in different environments.

QTL	Environments	Closest markers	LOD Score	R^2 (%of explanation)	Additive effect (A)
Test Weight	Kernen 2009	*Xwmc489*	3.62	13.0	0.44
		Sm1	3.54	12.7	0.42
	Kernen 2010	*Xwmc332*	2.01	7.50	-0.39
Thousand-Kernel Weight	Kernen 2009	*Xwmc489*	3.41	11.4	1.45
		Sm1	3.28	11.0	1.30
	Kernen 2010	*Sm1*	6.97	23.6	1.82
	Combined	*Xwmc489*	7.86	26.2	1.77
		Sm1	7.55	25.3	1.63
Falling Number	Kernen 2010	*Sm1*	2.37	8.80	7.69
Yield	Kernen 2009	*Sm1*	2.30	8.50	-19.9
		Xwmc489	1.98	7.40	-20.1
	Kernen 2010	*Sm1*	19.2	52.5	-59.2
		Xwmc489	13.0	39.6	-52.0
	Combined	*Sm1*	13.6	40.9	-39.5
		Xwmc489	9.96	32.0	-35.4
Yellow Pigment	Indian Head 2009	*Sm1*	2.89	10.6	-0.22
	Kernen 2009	*Sm1*	3.33	12.1	-0.29
		Xwmc489	2.91	10.6	-0.29
	Combined	*Sm1*	3.04	11.1	-0.24
		Xwmc489	2.51	9.20	-0.24
Grain Protein Content	Indian Head 2009	*Xwmc489*	2.11	7.80	0.24
		Sm1	1.76	6.60	0.22
	Kernen 2010	*2B_wPt-2106*	8.56	28.2	0.52
		Sm1	2.39	6.40	0.38
	Combined	*Sm1*	3.62	13.1	0.27
		Xwmc489	3.04	11.1	0.25
Wet Gluten Content	Kernen 2009	*Xwmc489*	2.60	9.40	-1.28
		Sm1	1.98	7.20	-1.11
	Kernen 2010	*2B_wPt-2106*	8.20	27.0	2.01
Gluten Index	Kernen 2009	*Xwmc489*	1.88	7.00	5.08
		Sm1	1.68	6.30	4.75
	Combined	*Xwmc489*	3.68	13.2	7.31
		Sm1	3.66	13.2	6.83
Stretching Time	Kernen 2010	*Sm1*	3.78	13.5	3.21
		Xwmc489	3.57	12.8	3.34
	Combined	*Sm1*	2.58	9.50	2.97

Additive effect (A): Additive effect of CDC Verona, (mu_A - mu_B)/2.
R^2: The phenotypic variation explained by the QTL.
LOD: Logarithm of odds.

5.0 Discussion

5.1 General Discussion

The grain quality of durum wheat is defined by its end products, predominantly pasta (Troccoli et al. 2000). Due to increased global pasta consumption, it is important to further enhance grain quality for industrial and nutritional purposes (El Ouafi et al. 2001). The breeding targets for high quality pasta durum wheat include consistent kernel size, high TWT, high GPC, high YP and strong gluten properties (Feillet and Dexter, 1996; Zhang et al. 2008; Sissons et al. 2005). However, damage to grain due to weather or insects is undesirable for appropriate end-use quality (Lunn et al. 1995; Oakley et al. 1998).

In order to prevent severe midge damage, the gene *Sm1*, which confers resistance to the OWBM, has been transferred into durum wheat from hexaploid wheat (Clarke et al. 2010). In this study, the introgression of *Sm1* was mapped to an approximately 11cM segment of chromosome 2BS in a $F_{5:9}$ RIL durum wheat mapping population. Midge resistant durum wheat cultivars have not yet been commercialized because breeding lines did not meet the strict quality requirements for the CWAD class. Therefore, the direct effects of *Sm1* on end-use quality of durum wheat need to be thoroughly studied. As detailed below, the presence of *Sm1* negatively impacted several agronomic and end-use quality traits. Thus, *Sm1* breeding strategies would have to account for these negative impacts of the *Sm1* introgression on end-use quality traits and strike an appropriate balance between end-use quality and resistance properties conferred by *Sm1*.

5.2 Pearson's Correlation Analysis of Agronomic and Grain Quality Traits

Mean data for the agronomic and end-use quality traits were analyzed to study the relationship among these traits. The high positive correlation ($r = 0.747$, $r=0.617$, $P< 0.001$, Table 8 and 9) between YP and GPC established in our study is in close

agreement with that reported by Samaan et al. (2006) and Dexter and Matsuo (1977). GPC may be associated with brownness in pasta (Borrelli et al. 1999; Dexter and Matsuo 1977; Taha and Sagi, 1986). However, high GPC in a big seed cannot guarantee high YP (Lacroix, 1974). TWT exhibited a negative correlation with YP ($r=-0.728$, $p<0.001$, Table 9), which may be because of dilution effects that reduced YP concentration when other grain constituents increased (Clarke et al. 2006; Hessler et al. 2002). Khattak et al. (2005) reported that TKW had negative correlations associated with GPC and gluten strength; however, no such correlations were observed in this study. In addition, TKW was not associated with most traits. Increased TKW may be explained as an indirect consequence of midge damage on kernels. A reduction in floret (and kernel) number would increase kernel size through assimilate accumulation and remobilization to the grain during grain filling (Suprayogi et al, 2009; People and Dalling, 1988; Feller and Fischer, 1994).

GPC was negatively correlated with YLD ($r=-0.669$, $P<0.001$, Table 9), in accordance with earlier published results (Stenram et al. 1990; Clarke et al. 2009). This could be due to the consequence of protein dilution when yield increased, or as the result of pleiotropic gene effects (Blanco et al. 2002). GPC was also negatively correlated with TWT ($r=-0.887$, $P<0.001$, Table 9). The negative correlation of GPC and TWT was likely associated with grain protein dilution in sprout kernels by OWBM infection, since damaged kernels often exhibit a split in the pericarp that exposes the embryo giving the kernels a sprouted appearance (Dexter et al. 1986).

ST were positively correlated with GI ($r=0.560$ and $r=0.523$, $p<0.001$, Table 8 and 9) and negatively correlated with RELAX ($r=-0.797$ and $r=-0.790$, $p<0.001$, Table 8 and 9). Strong gluten genotypes usually show longer ST with lower RELAX, a relationship which was also reported by Alamri et al. (2009).

A positive correlation ($r=0.945$, $p<0.001$, Table 9) between FN and TWT was observed in this study. FN was also positively correlated with YLD ($r=0.709$, $p<0.001$) (Table 9). In this study, reduced yield and FN could a result of sprout damage or midge damage.

5.3 Effects of Midge Damage on Seeds

The effects of *Sm1* in reducing midge damage on seeds cannot be completely proven in this study, since samples of the mapping population and check cultivars were only collected and analyzed at Kernen in 2010. Growing season precipitation was more than double the long-term average in 2010, so the growth of the durum plots was not typical. Little research exists on cultivars carrying the *Sm1* gene; nevertheless, *Sm1* appears effective in protecting the crop from large scale yield losses due to wheat midge infection (Lamb et al. 2000b). Generally, *Sm1* carriers revealed characteristics of OWBM resistance, evidenced by a smaller proportion of severely damaged seeds (Categories 3 and 4) and the opposite for categories 1 and 2 (Table 14). Positive significant genetic effects of *Sm1* on kernel physical appearance were demonstrated (Table 10 and 11), indicating that seed quality can be maintained by introducing the *Sm1* resistance gene. *Sm1* carriers tended to have better resistance to midge damage than 'CDC Verona' and 'DT780' (Table 11). 'Goodeve' hexaploid wheat, which carries *Sm1* and resistance to OWBM (Depauw et al. 2009), expressed similar characteristics to 'DT780'. But, 'Goodeve' exhibited a greater proportion of intact seeds (Category 1=61%) and did not reveal any significant difference from *Sm1* carriers (Table 11). There may still be some down-grading in cultivars carrying *Sm1* because the midge larvae need to feed briefly on the wheat kernels before they die (Lamb et al. 2000a). 'Waskada', without carrying *Sm1* but expressing antixenosis properties to deter egg-laying by OWBM (Fox et al. 2009). Moreover, under high midge pressure at Kernen 2010, 'Goodeve', 'Waskada', 'AC Avonlea', 'Eurostar' and 'Strongfield' all had the greater proportion of seeds in Categories 1 and 2, undamaged and slightly damaged seeds, respectively. It is possible that CWAD cultivars, 'AC Avonlea', 'Eurostar' and 'Strongfield' also expressed some midge tolerance properties to reduce seed damage from the OWBM.

5.4 Effect of *Sm1* on Agronomic and End-use Grain Quality Traits

5.4.1 Yield (YLD) and Test Weight (TWT), Thousand-kernel Weight (TKW)

Currently, there is little information linking the *Sm1* resistance gene to prevention of yield loss. However, it appears that *Sm1* is effective in protecting the crop from large scale yield loss (Lamb et al. 2000b). Compared to Kernen 2010 where OWBM pressure was high, higher yields were obtained from Kernen 2009 because of more favorable environmental conditions (Table 12). In addition, *Sm1* had a greater effect on yield in Kernen 2010 (39% higher yields from *Sm1* carriers at Kernen 2010 and 8% higher yields at Kernen 2009), compared to non-carriers (Table 12). Moreover, *Sm1* carriers had a higher yield than non-carriers in the combined analysis (Figure 6), indicating that *Sm1* had a positive effect on the expression of yield in this population. More midge resistance effects were observed in *Sm1* carriers when OWBM pressure was high in 2010. As expected, under different environmental conditions, lines carrying *Sm1* had higher yields than the lines and check cultivars lacking *Sm1* (Table 12). This suggests that *Sm1* reduced yield loss by preventing severe midge damage. Indeed, *Sm1* explained from 8.5% to 52.5% of the yield variation at Kernen 2010 (Table 17). In addition, the *Sm1* introgression had a positive influence on the expression of yield, probably contributed by other 'DT780' alleles. These results suggest that *Sm1* has a positive influence on the expression of yield and is a good resistance gene to prevent yield loss from midge damage.

In general, the expression of TWT is affected by growth environment, and has a moderate heritability (Bhatt and Derera, 1975; Jochum et al. 2001; Collaku and Harrison, 2005). Higher TWT was obtained from Kernen 2009 compared to Kernen 2010 where rainfall was high (Table 12). Wet weather conditions are known to reduce TWT (McCaig et al. 2006). In addition, elevated TWT was contributed by the 'CDC Verona' allele (Table 16 and Figure 6), while lines carrying *Sm1* had slightly lower values than 'CDC Verona' and *Sm1* non-carriers (Table 12). The QTL associated with TWT was flanked by *Sm1* and *Xgwm210*. These results suggest that the *Sm1* introgression just slightly reduced TWT in this mapping population.

The *Sm1* carriers had, on average, statistically lower TKW, however, not all lines carrying *Sm1* had a lower TKW (Figure 6). This provides some indication of finding lines that carry *Sm1* and exhibit higher TKW. QTLs associated with reduced TKW were detected by *Sm1* introgression, but elevated TKW was also contributed by 'CDC Verona' alleles on chromosome 2B (Table 17). However, small QTLs for TKW also were detected on the genetic map (Figure 11). Thus, complex genetic effects influenced the expressions of TKW, as reported by Clarke et al. (2009). Moreover, QTLs affecting TKW were also reported on chromosomes 1A, 2A, 2B, 3B, 4B, 5B, 6B and 7B (Houshmand et al. 2008). TKW could be influenced by other genetic effects, such as, the involvement of additive genetic effects (Ketata et al. 1976; Nachit et al. 1995). Therefore, it should be possible to overcome the negative effects of *Sm1* introgression on TKW by accumulating other favorable QTLs.

5.4.2 Falling Number (FN)

More than double the normal precipitation (average 48.8 mm/month) from May to September was recorded in 2010 (Table 13), in addition to significant rainfall, frost in September occurred during ripening. High midge pressure was also detected at Kernen 2010. Thus, extremely low FN was detected at Kernen 2010, compared with the other two environments (Table 14). The low FN values could be caused by pre-harvest sprouting or/and midge damage, as both are known to result in low FN. Pre-harvest sprouting refers to the precocious germination of the grain in the spike prior to harvest. Wet weather conditions lead to sprouted kernels of wheat (McCaig et al. 2006). Midge-damaged kernels also exhibit sprouting under poor weather conditions (Oakley, 1994; Fenney et al. 1988). Singh (2008) reported that more α-amylase activity from sprouted wheat samples results in a reduction in grain processing quality due to partial digestion of starch. Thus, more α-amylase activity from sprouted wheat grains causes lower FN. In addition, FN is usually negatively correlated with the proportion of damaged kernels (Helenius and Kurppa, 1989), suggesting that higher sprout damage and midge damage detected at Kernen 2010 causing the extremely low FN. 'Waskada', which is known to be more resistant to

pre-harvest sprouting and weathering damage (Fox et al. 2009), maintained a relatively high FN in all three environments (Table 14). Therefore, FN could be affected by both genotype and environment in this study.

Sm1 non-carriers expressed lower FN in the other two environments (Indian Head 2009 and Kernen 2009) compared to Kernen 2010, which could be due to the secretion of α-amylase by OWBM larva feeding on developing wheat kernels (Oakley et al. 1998). This suggests that *Sm1* may have had positive effects on the expression of FN by reducing the damage from OWBM. However, the opposite result was observed for one FN QTL at Kernen 2010; FN elevation associated with the *Sm1* introgression was contributed by the 'CDC Verona' allele (Figure 12 and Table 17). But, detection of this QTL may be due to the extremely low FN measurements at Kernen 2010. Therefore, it is not precise to conclude that FN was genetically influenced by the *Sm1* introgression.

5.4.3 Grain Protein Content (GPC)

GPC is strongly influenced by environment (Blanco et al. 2006; Mariani et al. 1995; Nachit et al. 1995; Trocoli et al. 2000). In the population used for this study, the effect of Environment*Entry was high for GPC (Table 7), indicating that the expression of GPC could be influenced by interaction of environmental and genetic effects. It was not surprising to observe higher GPC from the dramatically different environmental conditions at Kernen 2010 (Table 10). However, genetic effects overrode the environmental effects and had more influence on GPC (Table 3, 4, 5 and 7). This suggests that GPC is controlled by a complex genetic system.

Generally, good pasta cooking quality is related to a high GPC (D'Egidio et al. 1990; Ciaffi et al. 1991; Blanco et al. 1996; Malcolmson et al. 1993; El Ouafi, 2001). High GPC expression, above 13%, was measured for all check cultivars and the mapping population at Kernen 2009 and 2010 (Table 14). In addition, *Sm1* non-carriers had higher GPC than *Sm1* carriers (Table 14 and Figure 7). Similar results were also reported by Feillet and Dexter (1996), indicating that high GPC was the compensatory result of low grain yield caused by midge damage. However,

some *Sm1* carriers also expressed higher GPC (Figure 7), so lines that carry *Sm1* and express higher GPC can be expected in breeding populations.

In tetraploid wheat, QTLs for GPC are controlled by a complex genetic system (Blanco et al. 2006, Olmos et al. 2003; Gonzalez-Hernandez et al. 2004; Blanco et al. 2002). QTLs associated with GPC were identified on chromosomes 2B, 5B 6B and 7A in a doubled haploid durum wheat population (Suprayogi et al. 2009), and also found on 1B 2BS, 3BL, 4AL, 5AS, 5BL and 7AS in a RIL durum wheat population (Conti et al. 2011). In the present study, QTLs for GPC were also detected by *Sm1* introgression on chromosome 2B (Figure 12). However, the expression of the majority of these QTLs was environmentally dependent, and extremely significant at Kernen 2010. Similarly, moderate heritability with variable expression of QTL in different environments was also reported by Suprayogi et al. (2009).

In this study, positive alleles for elevated GPC at these loci were contributed by 'CDC Verona', thus *Sm1* introgression may result in reduced GPC (Table 17). 'Strongfield', a Canadian durum wheat cultivar (Clarke et al. 2005) not carrying *Sm1*, consistently displayed high GPC in the three environments that were part of this research (Table 14). Thus, 'Strongfield' is a good breeding parent for extensive durum wheat-crossing programs. These results suggest that it is possible to breed durum wheat cultivars for resistance to the OWBM while maintaining high GPC, by minimizing midge damage and linkage drag of the *Sm1* introgression. Genetic improvement of GPC and OWBM resistance is more economical and effective for durum wheat production than agronomic practices such as high nitrogen fertilization to improve GPC and insecticide application to protect against OWBM.

5.4.4 Yellow Pigment (YP)

YP was influenced by both environmental and genetic effects (Table 3, 4, 5 and 7), confirming the previous reports (Clarke et al. 2006; Ramachandran, 2010). More sprout-damaged samples were detected at Kernen 2010, however, some studies have concluded that sprout damage of wheat samples have little or no influence on YP

(Combe et al. 1988; Dick et al. 1974). However, in the mapping population used in this study, the expression of YP was environment dependent (Table 14). Conversely, YP expression was stable in the check cultivars across environments (Table 14). Significant genetic effects on YP were detected in individual environments (Table 3, 4 and 5). As reported, YP of durum wheat is highly heritable and is controlled by additive gene effects (Ramachandran, 2010; Reimer et al. 2008; Clarke et al. 2006; El Ouafi et al. 2001; Parker et al. 1998; Borreli et al. 1999; Nachit et al. 1995; Joppa and Williams, 1988a). Major genes controlling YP were reported on chromosomes 2A and 2B (Joppa and Williams, 1988a). In the present study, QTLs for YP were consistently detected by *Sm1* introgression on chromosome 2B (Figure 12). Moreover, *Sm1* carriers expressed higher YP (Figure 7) and elevated YP was contributed by the 'DT780' allele and 10.6% to 12.1% of variation was explained by *Sm1* (Table 17). These findings suggest that the *Sm1* introgression made a positive contribution to the expression of YP. Thus, it can be concluded that YP was mostly controlled by genetic effects.

5.4.5 Gluten Strength (GI, WETG, ST and RELAX)

In general, strong gluten cultivars usually show higher GI and longer ST than weak gluten cultivars (Alamri et al. 2009). As expected, 'Commander' and 'Brigade', both extra strong gluten durum cultivars, expressed significantly higher GI and higher ST than, 'AC Avonlea', a weak gluten variety (Table 15). The *Sm1* carriers expressed reduced gluten strength properties, with lower GI, shorter ST and lower WETG (Table 15); and moreover, *Sm1* carriers expressed reduced gluten strength (Figure 8). Therefore, it is evident that *Sm1* influences gluten strength properties.

Midge resistant lines carrying *Sm1* were expected to express better end-use grain quality as a result of superior dough properties, compared to *Sm1* non-carriers. It is well known that kernels exhibiting severe midge damage exhibit weak and sticky dough properties (Oakley, 1994; Fenney et al. 1988). Therefore, midge damage should have exerted a deleterious effect on durum wheat dough properties of *Sm1* non-carriers in this study. However, the opposite results were observed with *Sm1*

carriers expressing reduced gluten strength properties compared to non-carriers (Table 15). This could be due to insufficient removal of slightly damaged kernels (severely damaged kernels were eliminated prior to quality assessment) from the resistant lines during sampling, since slightly damaged kernel exhibited sprouting damage (Dexter et al. 1986). It is also possible that the kernel surfaces retained residual α-amylase enzyme from midge larva feeding on the developing wheat kernels (Oakley et al. 1998; Chandra et al. 2009).

Evaluation of seed damaged by midge infestation determined that *Sm1* carriers and non-carriers had 16.5 % and 12.8 % slightly damaged seeds (Category 2) (Table 11), respectively. Due to the possible presence of these damaged kernels in the samples, the enzyme α-amylase, as well as lipases and proteases may still remain in samples. These enzymes may be derived from either sprouted kernels (Lorenz and Valvano, 1981; Kruger, 1994) or midge infestation (Oakley et al. 1998; Chandra et al. 2009), thus reducing gluten strength properties through modification of amino acid composition (Oakley et al 1998; Lorenz and Valvano, 1981). In addition, damaged kernels may be more vulnerable to infestation by insects and contamination by pathogens and bacteria (Health Canada, 2006; Singh et al. 2008), resulting in poor quality meal for accurate evaluations of the traits studied.

In this study, QTLs for GI, WETG and ST were all associated with *Sm1* introgression on chromosome 2B. This is in accordance with the results reported by El Ouafi (2000) that gluten strength traits were localized on the following chromosome-arms: 1AS, 1BL, 2BS, 3AS, 3BS, 4BL, and 6BS. Stronger gluten properties were contributed by 'CDC Verona' alleles with the exception of WETG (Table 17). Moreover, lines expressing resistance to midge displayed reduced negative impacts from biotic stress on gluten properties. However, the results indicate that *Sm1* introgression had a negative impact on gluten properties in this study. This could be attributed to the sprout damage and midge damage at Kernen in 2010. Relatively wet conditions combined with high midge pressure occurred in this environment, resulting in weaker gluten properties than at Kernen in 2009 (Table 15). Wet conditions during the grain filling period were shown to have a negative

effect on gluten strength (Nachit et al. 1995; El Ouafi et al. 2000). Therefore, the apparent negative impact of *Sm1* introgression on gluten properties may be confounded with the effects of precipitation and midge damage in 2010. Favorable environment conditions and sufficient removal all damaged kernels (Categories 2, 3 and 4) prior to quality assessment would both help to eliminate errors in trait evaluation and examine the effect of *Sm1* on gluten properties.

5. 5 Analysis of Genetic Segregation

The observed segregation ratio of *Sm1* fit the expected 3:1 ratio ('CDC Verona' allele: 'DT780' allele) (Table 16). Three times more susceptible lines (*Sm1* "-") were detected from the final mapping population, which is abnormal segregation. Since both parents are homogeneous and homozygous, the reasons for explaining this phenomenon may be segregation distortion in meiosis (Lyttle, 1991). Segregation distortion may result from competition among gametes for preferential fertilization, as gametophyte genes expressed in the haploid gamete (Lyttle, 1991). The two parents in this study, 'DT780' (4×) derived from the hexaploid (6×) winter wheat, and 'CDC Verona' (4×) durum wheat, so that homologous chromosomes may not have paired well, or/and proper crossovers of homologous chromosome may not have happened during the meiotic phases. Thus, genetic differences among pollen from 'DT780' and 'CDC Verona' may have lead to gametophyte competition and selection, which resulted in nonrandom fertilization (Faris et al. 1998).

In addition, ten (59 %) loci (Chi square test, $P<0.05$) of these markers did not segregate in accordance with the expected Mendelian inheritance ratio of 1:1 (Table 16). The closest markers were *Xwmc489* and *Xgwm210*, which also showed 3:1 segregation distortion, suggesting *Sm1* was mapped as a single Mendelian factor along with the SSR, DArT and EST markers on chromosome 2B (Table 15). Moreover, Thomas et al. (2005) previously reported that *Sm1* is closely linked to *Xgwm210* and *Xwmc489* on chromosome 2BS. It is evident that *Sm1*, *Xgwm210* and *Xwmc489* all revealed 3:1 segregation distortion in this genetic background. Thus,

these two SSR markers are considered to be effective to detect *Sm1* loci on chromosome 2B.

The total size of the *Sm1* introgression was approximately 11cM (Figure 10, 11, 12 and 13). QTLs for damaged seed, YLD, FN, GPC and WETG were all detected by the region, which almost covered the entire *Sm1* introgression (Figure 10, 11, 12 and 13). However, there still was a 9.4cM interval between the *Sm1* locus and DArT maker 2B_wPt_2106 (Figure 10, 11, 12 and 13). This suggests that more efficient markers can be designed from this region and more phenotypic data may be required, in order to precisely detect QTLs associated with these traits. More efficient markers could help to reduce *Sm1* introgression, detect the *Sm1* locus, and enable a precise study of the effects of *Sm1* introgression or *Sm1* itself on these measured traits. TKW, TWT, YP, GI and ST were all flanked by *Sm1* and *Xwmc489*, involved in the *Sm1* introgression (Figure 10, 11, 12 and 13). This indicates that *Sm1* introgression significantly influenced the agronomic and end-use quality traits in the CDC Verona/DT780 mapping population. In the present study, *Sm1* and *Xwmc489* mapped 1.5cM apart (Figure 10, 11, 12 and 13). In contrast, in a previous study, these two loci mapped 6.3cM apart (Thomas et al. 2005). This means that even though *Xwmc489* is closely linked to *Sm1*, more work will be required to develop markers more closely linked, or co-segregating, with *Sm1*.

6.0 Conclusions and Future Work

A good understanding of the factors that influence pasta quality allows for breeding new durum wheat cultivars with improved end-use grain quality traits. In general, these include high GPC, FN, YP and stronger gluten properties. The hypothesis for this research was that there would be no direct influence of *Sm1* on end-use quality of durum wheat. However, contrary to this hypothesis, the presence of *Sm1* was associated with lower TKW, reduced GPC and weaker gluten properties. This could be due to the introgression with *Sm1* of genes that reduce end-use quality, or the results of pleiotropic effects of *Sm1* directly on end-use quality, or due to environmental effects on the expressions of these traits. Hence, *Sm1* breeding strategies would have to consider these negative impacts on end-use quality traits. However, some lines carrying *Sm1* expressed higher values of these important quality traits, suggesting the possibility of screening for lines that both carry *Sm1* and exhibit acceptable quality traits. On the other hand, *Sm1* had a positive effect on the expression of YLD and YP. Thus, the introduction of *Sm1* into durum wheat would help to reduce OWBM damage, which causes large scale yield loss, and improve YP.

Significant QTLs associated with *Sm1* were detected on chromosome 2B for TKW, TWT, YLD, YP, FN, GPC, GI, WETG, and RELAX. It should be noted that FN was quite low due to weather damage at Kernen 2010, thus only one QTL for FN was detected. QTLs associated with reduction in TKW, GPC and GI, WETG, and RELAX and were linked proximal to *Sm1*, indicating the possibility to breed better end-use quality by reducing the negative aspect of the *Sm1* introgression. From the present study, the total size of the *Sm1* introgression was approximately 11cM. *Sm1* was mapped as a single Mendelian factor on chromosome 2BS. *Sm1, Xwmc489,* and *Xgwm210* were closely linked and all showed segregation distortion deviating from the expected 1:1 ratio, resulting in an approximate 3:1 ratio towards susceptibility (CDC Verona allele).

According to the results from this thesis, the DNA markers reported here could be used to introgress midge resistance with desirable end-use quality traits into Canadian durum breeding germplasm. In order to reduce the linkage drag associated with *Sm1* introgression, efficient (ESTs, SSRs and DArT) markers should continue to be identified and developed. By increasing the marker density of the map, *Sm1* could be more precisely localized. In this study, the 2010 growing season was abnormal due to unusually high precipitation, which negatively impacted grain quality measurements. Thus, additional quality data should be collected to confirm the results observed here. Future research could also focus on identifying specific lines that carry *Sm1* and express desirable end-use quality traits. Other durum wheat populations could be developed that segregate for *Sm1*, and end-use quality traits could be studied in different genetic backgrounds. The line 'DT780' and other *Sm1* carriers have been used in additional crosses (Dr. Curtis Pozniak, personal communication), which could have result in the occurrence of smaller introgressed segments around *Sm1*. In this case, the direct effects of *Sm1* on the end-use quality of durum wheat could be thoroughly studied, which would aid in breeding midge-resistant durum wheat cultivars with desirable end-use quality and agronomic traits. In addition, CWAD cultivars, 'AC Avonlea', 'Strongfield' and 'Eurostar' also can be studied and investigated for potential midge resistant properties. Further characterization of possible antixenosis properties expressed by some check cultivars.

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8.0 Appendices

Appendix 1: Least significant difference (LSD) and least square means (LSM) of four categories of damaged seed for check cultivars and the CDC Verona/DT780 mapping population at Kernen2010.

		Damaged seeds			
Line	**S/R**	**Category 1 (%)**	**Category 2 (%)**	**Category 3 (%)**	**Category 4 (%)**
D05M80001	S	47.7	11.3	27.5	13.6
D05M80003	S	37.9	9.0	33.3	19.9
D05M80004	S	45.1	15.9	27.2	11.9
D05M80005	S	42.3	8.8	31.3	17.7
D05M80006	S	61.5	9.3	19.9	9.4
D05M80007	R	51.6	18.7	27.6	2.1
D05M80008	S	48.1	10.6	28.6	12.8
D05M80010	S	45.6	13.9	25.7	15.0
D05M80012	S	44.7	9.5	30.2	15.7
D05M80014	S	38.1	13.9	31.2	16.9
D05M80015	R	61.4	13.4	24.5	0.8
D05M80016	S	51.2	11.7	25.2	12.1
D05M80017	R	70.9	14.5	14.4	0.3
D05M80018	R	46.6	20.0	29.6	3.9
D05M80019	S	49.3	10.9	26.3	13.5
D05M80021	S	35.4	11.6	32.8	20.3
D05M80022	S	43.5	9.8	33.4	13.5
D05M80023	R	59.4	14.0	24.2	2.6
D05M80024	R	68.7	14.5	15.6	1.3
D05M80025	S	38.6	23.0	28.9	9.6
D05M80026	R	66.3	18.7	15.0	0.0
D05M80027	S	49.3	8.9	27.8	14.0
D05M80030	S	37.4	11.8	31.9	18.9
D05M80032	S	40.2	17.6	30.9	11.5
D05M80033	S	50.3	10.7	28.0	11.0
D05M80034	S	38.9	10.7	33.9	16.6
D05M80039	R	49.1	20.0	28.7	2.2
D05M80041	R	55.6	13.9	26.7	3.9
D05M80042	R	71.5	13.5	13.3	1.8
D05M80043	R	68.2	17.1	14.7	0.1
D05M80045	S	49.4	11.3	24.6	14.7
D05M80048	S	48.9	13.9	23.4	13.8
D05M80049	S	37.3	11.5	29.5	21.8
D05M80050	S	58.6	7.6	21.9	12.0
D05M80053	S	50.2	9.6	25.5	14.8
D05M80054	S	42.3	14.3	28.9	14.6
D05M80055	R	59.6	20.7	17.4	2.4
D05M80056	R	54.4	19.6	25.1	1.0
D05M80057	S	31.3	22.4	26.9	19.5
D05M80058	S	58.2	14.4	17.8	9.7
D05M80059	R	56.5	16.3	26.9	0.4
D05M80060	S	31.5	19.2	35.8	13.5
D05M80063	S	50.2	9.9	25.2	14.9
D05M80064	S	61.1	11.3	20.1	7.6
D05M80067	S	42.7	13.1	31.9	12.5

D05M80068	R	70.4	16.6	12.5	0.6
D05M80069	S	44.2	11.6	26.7	17.6
D05M80070	S	44.1	11.9	33.3	10.9
D05M80071	R	73.9	12.0	13.7	0.5
D05M80074	S	46.6	10.7	26.3	16.6
D05M80076	S	50.2	11.1	29.2	9.6
D05M80077	R	46.0	14.5	36.8	2.8
D05M80078	R	54.1	24.3	18.9	2.8
D05M80079	S	58.7	11.2	24.0	6.2
D05M80080	R	57.3	15.4	25.4	2.0
D05M80081	S	54.3	11.3	23.4	11.0
D05M80082	R	33.9	18.9	40.9	6.4
D05M80083	R	25.3	21.0	50.6	3.1
D05M80084	S	56.2	10.6	21.2	12.1
D05M80086	S	46.0	18.2	26.2	9.7
D05M80087	S	54.7	10.4	25.3	9.7
D05M80088	S	44.2	13.3	29.9	12.8
D05M80091	R	48.9	11.2	39.3	0.8
D05M80092	R	63.8	14.3	19.7	2.3
D05M80095	S	36.3	27.1	27.0	9.6
D05M80097	S	45.1	17.4	34.6	3.0
D05M80098	S	45.7	12.1	26.2	16.1
D05M80100	R	56.6	16.6	25.8	1.1
D05M80101	S	44.6	10.8	27.4	17.4
D05M80102	R	69.3	14.4	16.1	0.3
D05M80103	S	41.6	14.4	30.2	13.8
D05M80105	S	44.3	13.1	30.2	12.5
D05M80107	S	48.0	12.8	26.9	12.3
D05M80108	S	35.0	11.1	33.3	20.7
D05M80109	S	55.7	9.9	22.1	12.4
D05M80110	S	52.8	12.5	24.5	10.3
D05M80112	S	41.6	17.4	28.9	12.2
D05M80117	S	41.0	8.7	31.5	18.9
D05M80119	R	54.5	13.4	27.4	4.8
D05M80120	S	37.0	14.9	34.6	13.7
D05M80121	S	45.3	10.8	28.0	15.9
D05M80122	R	63.2	22.5	14.4	0.0
D05M80123	S	38.3	18.1	25.2	18.5
D05M80125	S	47.7	10.7	28.4	13.4
D05M80127	S	44.1	12.8	20.5	22.7
D05M80129	S	50.7	12.1	27.9	9.3
D05M80130	S	44.8	11.5	30.0	13.8
D05M80131	S	41.0	8.2	32.4	18.6
D05M80134	R	57.3	12.9	27.4	2.4
D05M80135	S	58.5	11.2	19.0	11.3
D05M80137	S	59.5	12.0	17.4	11.2
D05M80140	S	51.9	15.9	19.9	12.3
D05M80141	S	31.1	18.1	31.6	19.4
D05M80142	S	45.0	13.5	25.6	15.9
D05M80143	R	43.6	18.0	34.3	4.2
D05M80146	S	45.1	11.4	28.6	15.0
D05M80147	S	58.8	10.6	19.7	11.0
D05M80148	S	59.5	10.8	21.5	8.4
D05M80149	S	50.2	9.7	26.4	13.8
D05M80151	S	37.2	10.4	30.2	22.3
D05M80154	S	56.2	12.2	23.2	8.5

D05M80155	S	41.2	13.3	29.3	16.3
D05M80156	R	39.6	21.3	34.4	4.9
D05M80157	S	45.4	12.3	27.2	15.2
D05M80158	S	44.9	15.5	29.6	10.1
D05M80160	S	44.9	11.5	29.8	13.9
Goodeve	R	60.6	13.1	16.2	10.2
Waskada	S	49.7	21.1	16.2	13.0
ACAvonlea	S	56.6	10.8	22.5	10.2
ACMorse	S	40.3	18.4	29.7	11.8
ACNavigator	S	39.5	14.8	36.4	9.4
Brigade	S	41.0	20.2	29.5	9.5
Commander	S	31.6	18.8	33.5	16.2
Eurostar	S	51.0	10.0	24.8	14.2
Strongfield	S	50.2	17.9	24.2	7.7
CDCVerona	S	39.4	12.0	30.3	18.4
DT780	R	46.1	13.7	29.8	10.5
Mean (pop.)		48.9	14.0	26.6	10.6
Max (pop.)		73.9	27.1	50.6	22.7
Min (pop.)		25.3	7.60	12.5	0.0
LSD $_{0.05}$		16.0	7.70	10.5	7.50

Appendix 2: Least significant difference (LSD) and least square means (LSM) of heading date (HD), plant height (PH), yield (YLD), test weight (TWT), thousand-kernel weight (TKW), falling number (FN), grain protein content (GPC) for check cultivars and the CDC Verona/DT780 mapping population for three environments (Indian Head 2009, Kernen 2009 and Kernen 2010).

		HD (day)			PH (cm)			YLD (kg/ha)			TWT (kg/hl)			TKW (g)			FN (sec)				GPC (%)			
Line	**S/R**	KN09	KN10	COMBINED	KN09	KN10	COMBINED	KN09	KN10	COMBINED	KN09	KN10	COMBINED	KN09	KN10	COMBINED	IH09	KN09	KN10	COMBINED	IH09	KN09	KN10	COMBINED
D05M80001	S	58.5	59.5	59.0	94.5	98.0	96.3	4715	2663	3689	82.0	74.5	78.3	44.7	46.9	45.8	398	357	86	280	12.6	15.7	19.5	15.9
D05M80002	S	59.0	58.5	58.8	90.5	89.5	90.0	5348	4070	4709	82.4	74.6	78.4	42.6	50.2	46.4	412	356	62	276	10.4	14.9	18.6	14.6
D05M80003	S	59.0	57.5	58.3	93.0	98.5	95.8	5169	3114	4141	82.8	74.6	78.7	50.3	50.2	50.2	400	348	132	293	11.9	14.2	18.5	14.8
D05M80004	S	58.5	57.5	58.0	82.0	86.5	84.3	4593	2998	3796	80.6	74.1	77.3	44.2	48.3	46.3	431	408	78	305	12.0	13.6	19.5	15.0
D05M80005	S	60.5	60.0	60.3	97.5	97.5	97.5	4946	2463	3705	83.5	74.6	79.0	47.7	48.6	48.1	377	322	75	258	11.7	14.4	19.2	15.1
D05M80006	S	59.5	58.0	58.8	97.0	83.0	90.0	5910	2737	4323	82.1	77.8	79.9	42.3	44.6	43.4	441	392	122	318	11.4	13.3	17.8	14.1
D05M80007	R	61.0	59.5	60.3	91.5	100.0	95.8	5169	3653	4411	82.4	74.3	78.3	49.0	43.8	46.4	396	341	66	268	11.5	14.0	17.9	14.5
D05M80008	S	59.5	60.0	59.8	98.5	99.0	98.8	5790	3511	4650	83.1	75.3	79.2	51.2	49.7	50.4	401	345	74	273	13.2	15.8	18.3	15.8
D05M80010	S	60.5	58.5	59.5	99.0	102.0	100.5	5061	2490	3775	81.3	72.2	76.8	42.6	43.4	43.0	471	383	68	307	13.0	15.0	19.9	15.9
D05M80012	S	60.0	58.5	59.3	92.0	85.0	88.5	4611	2424	3518	83.1	73.3	78.2	51.8	49.3	50.5	396	340	104	280	11.3	14.4	18.9	14.8
D05M80014	S	60.0	59.0	59.5	93.0	99.5	96.3	4192	2862	3527	82.1	70.8	76.4	46.8	48.3	47.5	393	316	62	257	12.5	14.2	20.5	15.7
D05M80015	R	59.5	59.5	59.5	95.0	92.5	93.8	5721	4451	5086	82.5	73.9	78.2	44.2	44.4	44.3	357	315	62	245	11.6	14.2	17.3	14.3
D05M80016	S	58.5	59.5	59.0	88.5	93.0	90.8	5797	4074	4935	82.2	74.5	78.3	42.9	44.6	43.8	402	331	65	266	10.9	12.9	18.3	14.0
D05M80017	R	61.0	59.0	60.0	103.5	98.5	101.0	6033	4358	5195	81.1	75.9	78.5	44.8	46.3	45.5	459	391	95	315	10.9	14.6	17.4	14.3
D05M80018	R	60.0	58.5	59.3	90.5	89.5	90.0	5234	4142	4688	82.0	74.6	78.3	43.3	44.2	43.7	434	328	81	281	11.6	14.9	18.7	15.0
D05M80019	S	60.5	60.0	60.3	98.5	100.5	99.5	5155	4566	4861	82.5	74.7	78.6	48.1	55.4	51.8	362	319	62	248	11.6	14.8	18.1	14.8
D05M80021	S	60.0	58.5	59.3	94.5	93.0	93.8	5266	3091	4178	83.5	74.3	78.9	45.0	50.2	47.6	438	375	86	300	11.9	14.5	19.9	15.4
D05M80022	S	60.5	59.5	60.0	103.0	94.0	98.5	5045	2667	3856	82.7	72.6	77.6	45.7	45.3	45.5	435	366	65	288	11.6	14.1	19.3	15.0
D05M80023	R	59.0	58.0	58.5	91.5	82.5	87.0	4196	3556	3876	83.3	74.3	78.8	45.0	45.9	45.4	391	355	64	270	10.6	12.6	16.7	13.3

D05M80024	R	58.0	57.5	57.8	89.5	97.0	93.3	5169	3980	4574	82.1	76.3	79.2	39.1	43.0	41.0	390	345	89	274	11.4	13.2	16.3	13.6
D05M80025	S	57.5	56.5	57.0	90.0	84.0	87.0	3520	3255	3388	82.4	72.8	77.6	46.8	50.8	48.8	382	315	85	261	11.9	14.4	18.7	15.0
D05M80026	R	58.0	58.0	58.0	100.5	81.5	91.0	5462	3975	4718	81.3	73.5	77.4	41.8	41.6	41.7	462	387	94	314	11.4	14.4	18.3	14.7
D05M80027	S	58.5	59.5	59.0	93.0	102.0	97.5	4202	3037	3619	84.3	75.0	79.6	45.3	45.5	45.4	416	361	130	302	13.1	12.8	18.8	14.9
D05M80028	S	58.5	58.0	58.3	90.5	91.5	91.0	4316	3179	3747	82.6	71.3	77.0	44.6	51.2	47.9	392	316	72	260	11.7	13.7	18.7	14.7
D05M80030	S	60.5	60.0	60.3	92.5	104.0	98.3	4835	2928	3881	82.4	74.8	78.6	43.3	44.1	43.7	425	361	77	288	12.6	14.7	19.9	15.7
D05M80032	S	57.5	58.0	57.8	94.0	95.5	94.8	4861	3724	4293	82.1	73.5	77.8	50.7	53.5	52.1	368	297	82	249	12.2	13.2	17.4	14.3
D05M80033	S	59.5	59.0	59.3	93.5	99.0	96.3	6024	3887	4955	83.1	74.7	78.9	48.9	50.9	49.9	436	389	151	325	12.1	14.9	18.4	15.1
D05M80034	S	60.0	59.5	59.8	95.0	94.5	94.8	5128	2585	3856	83.4	74.4	78.9	53.3	49.1	51.2	388	349	117	285	11.8	14.6	18.6	15.0
D05M80039	R	57.5	57.5	57.5	82.0	88.5	85.3	4733	3976	4354	82.1	73.3	77.7	44.9	44.1	44.5	395	340	63	266	12.0	14.2	18.4	14.8
D05M80041	R	60.0	58.5	59.3	89.5	88.0	88.8	5675	4677	5176	82.8	73.1	77.9	50.8	46.6	48.7	386	341	62	263	11.1	13.8	16.7	13.8
D05M80042	R	60.5	58.5	59.5	93.5	88.0	90.8	5862	4214	5038	83.3	76.1	79.7	46.4	44.2	45.3	402	354	81	279	10.3	13.8	15.5	13.2
D05M80043	R	59.5	58.5	59.0	96.0	103.5	99.8	3871	4338	4104	81.1	73.0	77.0	46.4	45.1	45.7	391	337	62	263	12.5	14.4	18.4	15.1
D05M80045	S	58.5	58.0	58.3	84.5	91.5	88.0	4445	3070	3757	83.0	72.9	77.9	46.4	50.1	48.2	373	311	63	249	12.2	13.4	19.0	14.9
D05M80048	S	60.5	60.5	60.5	96.5	99.0	97.8	5887	2733	4310	82.4	74.3	78.3	50.9	50.9	50.9	348	317	73	246	11.1	14.8	19.0	14.9
D05M80049	S	60.5	60.0	60.3	90.0	97.5	93.8	5354	1920	3637	82.3	73.5	77.9	48.7	49.1	48.9	408	329	73	270	12.3	14.3	19.8	15.5
D05M80050	S	59.5	59.0	59.3	93.5	89.5	91.5	5233	3025	4129	83.1	75.7	79.4	44.2	48.6	46.4	463	376	113	317	11.9	14.1	19.1	15.0
D05M80053	S	60.5	59.5	60.0	95.5	95.5	95.5	4692	3122	3907	84.0	73.6	78.8	50.9	49.5	50.2	419	346	63	276	10.5	13.0	17.9	13.8
D05M80054	S	58.5	59.0	58.8	86.0	92.5	89.3	4485	3148	3817	82.6	73.2	77.9	45.8	45.2	45.5	437	295	69	267	12.9	15.6	18.8	15.8
D05M80055	R	60.0	59.5	59.8	95.0	101.5	98.3	6216	4787	5501	81.4	74.6	78.0	44.7	44.1	44.4	415	374	64	284	12.6	14.7	17.7	15.0
D05M80056	R	59.5	57.5	58.5	94.0	91.5	92.8	5471	4414	4942	83.0	76.5	79.8	42.6	42.6	42.6	445	396	82	308	11.7	15.2	18.0	15.0
D05M80057	S	60.0	60.0	60.0	93.0	94.0	93.5	5010	3074	4042	84.0	74.5	79.2	45.3	45.5	45.4	396	355	103	284	12.1	14.1	19.5	15.2
D05M80058	S	60.0	58.0	59.0	94.5	94.0	94.3	4896	4617	4757	82.6	74.2	78.4	47.1	46.3	46.7	404	339	77	273	10.4	13.6	18.0	14.0
D05M80059	R	58.5	57.5	58.0	85.5	84.5	85.0	5203	4064	4633	82.0	72.6	77.3	46.6	45.0	45.8	459	360	63	294	12.5	16.7	18.4	15.8
D05M80060	S	59.0	58.0	58.5	86.5	78.0	82.3	5014	1774	3394	83.0	75.1	79.0	46.6	42.5	44.5	388	334	100	274	12.5	15.2	18.8	15.5
D05M80063	S	58.5	59.0	58.8	87.0	99.0	93.0	4848	3457	4152	82.5	75.2	78.8	44.4	49.5	46.9	433	366	70	289	11.4	13.6	19.8	14.9
D05M80064	S	56.5	56.0	56.3	80.5	83.5	82.0	4107	3311	3709	81.7	73.8	77.7	41.8	43.5	42.6	427	371	133	310	13.3	15.5	19.6	16.1
D05M80067	S	59.5	59.0	59.3	94.5	86.5	90.5	5422	2231	3826	83.3	73.3	78.3	46.6	42.7	44.6	391	329	95	271	13.1	15.4	19.8	16.1
D05M80068	R	59.5	57.5	58.5	95.0	86.5	90.8	4655	4560	4607	80.2	74.8	77.5	40.0	42.3	41.1	425	386	70	293	11.8	16.2	18.0	15.3
D05M80069	S	60.5	59.5	60.0	91.0	96.5	93.8	5388	3134	4261	84.2	74.1	79.1	49.6	49.3	49.4	400	337	77	271	11.9	13.6	19.4	14.9

D05M80070	S	60.5	59.0	59.8	88.0	92.5	90.3	4396	3218	3807	82.6	73.4	78.0	51.2	47.2	49.2	379	295	83	252	12.3	14.4	18.6	15.1
D05M80071	R	59.0	57.0	58.0	89.5	84.0	86.8	4630	4482	4556	80.9	75.0	77.9	41.4	42.3	41.8	422	368	82	291	12.2	14.4	18.1	14.9
D05M80074	S	60.5	59.5	60.0	94.0	87.5	90.8	4922	2455	3688	83.4	75.5	79.5	50.9	47.0	48.9	333	317	87	245	13.0	14.2	17.8	15.0
D05M80076	S	59.5	58.5	59.0	93.0	93.5	93.3	5015	2895	3955	82.9	74.5	78.7	46.6	46.7	46.7	452	365	164	327	11.8	14.0	19.0	14.9
D05M80077	R	60.0	59.0	59.5	94.0	97.0	95.5	5826	3772	4799	81.8	73.4	77.6	45.0	46.7	45.8	392	335	63	263	10.8	13.7	17.5	14.0
D05M80078	R	58.5	57.0	57.8	90.5	90.5	90.5	6050	4554	5302	82.8	76.0	79.4	41.9	43.5	42.7	413	340	63	272	12.4	15.0	17.8	15.1
D05M80079	S	60.0	58.0	59.0	86.0	81.0	83.5	4501	2778	3640	82.2	75.1	78.6	50.6	47.7	49.1	365	308	116	263	11.9	14.9	16.6	14.5
D05M80080	R	61.0	59.5	60.3	93.0	97.0	95.0	5293	4655	4974	82.0	74.2	78.1	46.7	47.6	47.1	384	327	63	258	11.3	14.1	17.6	14.3
D05M80081	S	60.0	58.5	59.3	89.5	95.0	92.3	4665	3253	3959	83.2	76.0	79.6	44.8	43.4	44.1	403	342	118	287	12.1	14.2	18.4	14.9
D05M80082	R	58.0	60.0	59.0	86.5	94.5	90.5	3578	4111	3845	82.9	73.2	78.0	48.4	45.7	47.0	393	293	62	249	12.1	14.0	17.6	14.6
D05M80083	R	60.5	60.5	60.5	92.0	95.5	93.8	6593	3546	5069	82.4	71.9	77.2	47.3	44.2	45.7	372	319	62	251	10.8	14.5	18.0	14.4
D05M80084	S	57.5	58.0	57.8	87.5	83.0	85.3	4827	3231	4029	81.9	74.5	78.2	43.1	48.4	45.7	426	355	66	282	11.7	14.8	19.0	15.2
D05M80086	S	58.5	57.0	57.8	90.0	86.0	88.0	4769	3009	3889	83.7	74.2	79.0	49.9	48.9	49.4	365	336	89	263	12.6	13.6	18.8	15.0
D05M80087	S	60.0	58.5	59.3	97.5	93.5	95.5	5949	3527	4738	84.4	75.8	80.1	45.5	45.9	45.7	424	389	67	293	10.7	14.2	18.9	14.6
D05M80088	S	60.0	59.0	59.5	91.0	91.0	91.0	4571	2175	3373	83.5	74.4	78.9	47.2	44.7	45.9	400	319	72	264	12.0	15.2	19.8	15.6
D05M80089	S	60.0	59.0	59.5	99.0	99.0	99.0	5712	3562	4637	83.4	76.0	79.7	50.7	50.3	50.5	394	357	90	280	11.1	14.7	17.9	14.6
D05M80090	S	59.0	58.0	58.5	91.0	88.5	89.8	4781	2523	3652	85.1	71.3	78.2	46.4	46.9	46.6	325	260	62	215	12.0	14.0	18.1	14.7
D05M80091	R	60.0	60.0	60.0	92.0	95.0	93.5	5361	4408	4884	84.0	74.3	79.1	49.7	47.2	48.4	382	330	62	258	11.6	13.6	18.1	14.4
D05M80092	R	59.0	59.0	59.0	95.0	101.0	98.0	5772	4179	4975	82.0	73.9	78.0	50.3	45.3	47.8	425	379	69	291	11.2	15.5	18.3	15.0
D05M80095	S	60.0	58.5	59.3	88.0	82.5	85.3	4473	3725	4099	80.9	73.2	77.1	47.3	49.6	48.4	336	308	65	236	11.8	13.7	18.1	14.5
D05M80096	S	61.5	61.0	61.3	95.0	99.0	97.0	4852	3058	3955	82.4	74.6	78.5	42.9	47.6	45.2	431	358	118	302	12.3	13.2	18.0	14.5
D05M80097	S	58.5	58.0	58.3	84.0	78.5	81.3	4080	2381	3230	81.8	72.9	77.3	45.8	40.7	43.2	379	355	62	265	13.0	15.3	18.9	15.7
D05M80098	S	59.5	59.0	59.3	97.5	104.5	101.0	5017	3972	4494	81.6	74.8	78.2	44.0	52.2	48.1	470	353	81	301	10.8	13.2	19.6	14.5
D05M80100	R	59.0	59.5	59.3	81.5	90.5	86.0	5311	4340	4826	83.3	70.3	76.8	47.4	45.1	46.2	383	324	62	256	11.4	14.0	18.2	14.5
D05M80101	S	59.0	58.5	58.8	83.5	93.5	88.5	3738	2979	3358	82.1	74.9	78.4	46.1	46.1	46.1	409	351	68	276	12.5	14.4	19.0	15.3
D05M80102	R	60.0	59.5	59.8	88.5	91.0	89.8	6273	4916	5594	82.4	73.7	78.0	47.4	44.6	46.0	386	364	61	270	12.0	14.5	17.9	14.8
D05M80103	S	57.0	57.5	57.3	84.0	89.5	86.8	4474	3362	3918	81.6	74.9	78.3	46.6	48.8	47.7	413	369	74	285	11.9	13.9	19.6	15.1
D05M80104	S	58.5	59.0	58.8	91.0	89.5	90.3	5076	2910	3993	83.8	73.0	78.4	45.0	46.9	45.9	418	316	63	266	12.5	14.8	19.6	15.6
D05M80105	S	59.5	58.0	58.8	92.0	97.0	94.5	4459	3447	3953	83.5	74.2	78.8	48.8	51.6	50.2	386	313	83	261	12.1	13.7	18.4	14.7
D05M80107	S	56.5	56.5	56.5	83.5	81.5	82.5	4975	2653	3814	83.7	74.3	79.0	46.0	47.7	46.8	408	341	162	304	12.7	15.1	18.8	15.5

D05M80108	S	59.5	59.5	59.5	92.0	102.0	97.0	4767	2650	3708	83.3	72.4	77.8	48.0	50.2	49.1	441	367	76	294	12.1	14.1	20.9	15.7
D05M80109	S	61.0	59.5	60.3	96.0	94.0	95.0	5106	3255	4180	83.8	74.6	79.2	47.4	47.7	47.5	396	353	78	275	10.7	13.0	18.6	14.1
D05M80110	S	58.5	58.0	58.3	84.0	87.0	85.5	5187	3031	4109	80.7	72.0	76.3	45.5	46.5	46.0	406	320	70	265	12.6	14.6	19.8	15.6
D05M80112	S	58.0	59.0	58.5	87.5	100.5	94.0	4878	3426	4152	82.0	73.7	77.8	47.3	48.6	47.9	423	336	96	285	11.9	15.2	20.7	15.9
D05M80116	S	61.5	60.5	61.0	92.0	105.5	98.8	4625	3545	4085	82.5	74.9	78.7	49.2	46.9	48.0	378	312	92	260	12.3	15.1	18.0	15.1
D05M80117	S	59.5	59.5	59.5	92.0	94.5	93.3	5057	2455	3756	83.6	73.1	78.4	52.1	48.6	50.3	428	351	128	302	12.0	16.4	19.5	15.9
D05M80119	R	59.0	58.5	58.8	86.5	100.5	93.5	5292	4609	4951	82.7	75.6	79.1	45.7	46.4	46.0	406	358	63	276	11.4	13.9	17.3	14.2
D05M80120	S	59.0	58.0	58.5	88.0	95.0	91.5	4623	2753	3688	82.6	72.6	77.6	49.3	46.0	47.6	389	304	65	253	12.6	16.8	19.8	16.4
D05M80121	S	59.5	59.5	59.5	86.5	92.0	89.3	4761	3095	3928	82.9	74.0	78.5	48.6	48.0	48.3	318	301	62	227	12.0	13.3	18.4	14.5
D05M80122	R	57.5	57.5	57.5	87.0	89.5	88.3	5519	3677	4598	82.2	74.6	78.4	43.9	41.7	42.8	432	389	66	296	12.1	16.2	18.2	15.5
D05M80123	S	59.5	59.0	59.3	93.5	100.0	96.8	5015	3846	4430	83.4	75.6	79.5	46.1	49.5	47.8	366	303	96	255	14.1	15.2	19.5	16.3
D05M80124	S	59.0	57.5	58.3	83.5	93.5	88.5	4211	2953	3582	81.7	73.5	77.6	47.0	47.0	47.0	398	312	74	261	13.3	15.1	19.0	15.8
D05M80125	S	59.0	59.0	59.0	86.5	88.0	87.3	4743	2675	3709	83.1	74.1	78.6	49.4	47.1	48.2	467	349	82	299	12.6	14.4	19.8	15.6
D05M80126	R	59.5	58.0	58.8	97.0	99.5	98.3	5172	4140	4656	81.9	74.7	78.3	45.9	47.7	46.8	425	356	91	290	12.9	15.9	19.0	15.9
D05M80127	S	58.5	61.0	59.8	83.5	92.5	88.0	4495	3126	3810	83.6	73.2	78.4	46.9	50.2	48.5	387	344	92	274	12.3	13.9	18.8	15.0
D05M80129	S	60.5	59.5	60.0	94.5	96.0	95.3	5247	2883	4065	84.0	75.2	79.6	50.2	49.6	49.9	379	328	83	263	11.1	13.8	17.9	14.2
D05M80130	S	60.0	58.5	59.3	97.5	100.5	99.0	5308	3976	4642	83.3	74.9	79.1	44.9	50.3	47.6	433	362	69	288	11.0	13.3	19.2	14.5
D05M80131	S	61.5	60.0	60.8	99.0	103.5	101.3	5624	2780	4202	83.9	75.0	79.4	48.9	47.1	48.0	414	380	67	287	11.6	15.2	20.8	15.8
D05M80134	R	59.5	57.5	58.5	99.5	96.5	98.0	5126	3558	4342	81.9	72.8	77.3	47.5	43.5	45.5	421	350	73	281	12.2	15.8	18.3	15.4
D05M80135	S	60.0	59.5	59.8	94.5	103.5	99.0	5869	3630	4750	81.7	75.8	78.7	44.8	48.2	46.5	417	335	157	303	11.3	15.2	18.3	14.9
D05M80136	S	61.0	58.0	59.5	90.5	85.5	88.0	4835	2620	3727	83.8	75.3	79.5	45.1	41.2	43.2	433	333	93	286	13.2	15.9	18.7	15.9
D05M80137	S	59.5	57.5	58.5	90.5	81.0	85.8	5632	3560	4596	83.2	75.8	79.5	41.2	42.7	41.9	364	320	66	250	11.8	14.1	17.7	14.5
D05M80140	S	58.0	57.5	57.8	83.5	88.0	85.8	4765	3058	3911	83.3	75.3	79.3	47.1	49.1	48.1	443	358	86	295	13.5	14.9	19.3	15.9
D05M80141	S	59.0	59.0	59.0	91.0	92.0	91.5	4478	2523	3500	82.6	73.6	78.1	50.9	47.0	49.0	424	326	98	282	11.2	16.6	19.0	15.6
D05M80142	S	59.5	59.5	59.5	91.0	99.5	95.3	4065	2659	3362	83.2	72.3	77.7	47.4	43.4	45.4	383	325	66	258	12.0	13.6	19.9	15.1
D05M80143	R	59.5	58.5	59.0	97.5	104.5	101.0	4497	3630	4063	83.8	75.8	79.8	45.7	43.7	44.7	368	317	63	249	12.6	14.6	18.2	15.1
D05M80144	R	56.5	57.5	57.0	88.0	101.0	94.5	6107	5307	5707	81.5	76.4	78.9	38.9	44.2	41.5	434	410	87	310	12.1	13.5	17.1	14.2
D05M80146	S	61.0	60.0	60.5	95.0	103.0	99.0	5742	3002	4372	80.6	71.8	76.2	44.4	42.8	43.6	451	310	62	274	12.8	17.2	20.7	16.9
D05M80147	S	57.5	57.5	57.5	89.0	97.5	93.3	5355	3476	4415	83.0	75.2	79.1	46.3	49.9	48.1	453	355	66	291	12.0	15.8	19.8	15.8
D05M80148	S	59.5	57.5	58.5	88.0	76.0	82.0	4782	2743	3763	83.0	73.1	78.0	48.7	45.1	46.9	339	336	68	247	12.0	14.4	18.0	14.8

D05M80149	S	58.5	59.0	58.8	94.5	97.0	95.8	5186	2515	3850	84.3	75.4	79.8	48.3	42.7	45.5	387	338	131	285	12.9	14.9	18.1	15.3
D05M80151	S	60.5	59.5	60.0	92.5	95.5	94.0	5486	3261	4374	83.6	73.5	78.5	50.5	51.2	50.8	361	307	63	243	12.6	14.8	19.6	15.7
D05M80154	S	59.0	57.5	58.3	94.0	92.0	93.0	5445	2496	3970	82.5	75.3	78.9	48.4	45.8	47.1	362	330	87	259	12.3	16.8	18.4	15.8
D05M80155	S	59.5	59.5	59.5	92.5	96.0	94.3	4198	2439	3318	83.5	74.5	79.0	44.3	44.7	44.5	412	367	82	287	13.8	14.7	20.3	16.2
D05M80156	R	60.5	60.0	60.3	98.0	103.0	100.5	4870	4239	4554	81.4	73.9	77.6	42.6	43.9	43.2	434	412	62	302	10.7	14.6	18.0	14.4
D05M80157	S	60.5	58.0	59.3	95.0	85.0	90.0	4767	2673	3720	81.4	73.3	77.3	45.1	43.4	44.2	400	355	92	282	12.5	15.1	18.7	15.4
D05M80158	S	60.0	57.5	58.8	87.5	83.0	85.3	3909	2538	3223	81.1	72.8	76.9	47.4	46.3	46.9	396	344	71	270	12.6	14.3	19.1	15.3
D05M80159	S	58.5	57.5	58.0	88.0	87.5	87.8	4758	2961	3859	82.2	73.8	78.0	43.4	44.8	44.1	399	346	76	273	11.9	13.3	18.6	14.6
D05M80160	S	59.0	59.0	59.0	91.0	97.5	94.3	5588	2959	4274	83.3	73.8	78.5	47.4	49.4	48.4	366	315	62	248	11.8	15.4	19.0	15.4
Goodeve	R	56.0	57.0	56.5	88.5	98.5	93.5	3210	3792	3501	80.5	78.4	79.4	36.2	43.7	40.0	431	390	114	312	13.2	14.5	19.4	15.7
Waskada	S	56.0	55.5	55.8	96.0	101.0	98.5	4796	3087	3941	82.6	79.5	81.0	37.3	38.6	37.9	391	381	317	363	13.8	15.6	19.4	16.2
ACAvonlea	S	58.5	56.5	57.5	92.5	81.5	87.0	3699	1817	2758	82.0	75.5	78.8	45.4	44.5	45.0	393	353	95	280	12.7	15.0	19.4	15.7
ACMorse	S	58.5	58.0	58.3	87.5	91.5	89.5	4669	3441	4055	81.5	73.9	77.7	48.2	46.4	47.3	462	364	133	319	11.8	14.6	18.6	15.0
ACNavigator	S	62.0	56.5	59.3	78.5	80.5	79.5	4468	2431	3449	83.6	70.7	77.1	49.3	48.7	49.0	475	352	73	300	11.8	13.8	18.1	14.6
Brigade	S	62.5	60.0	61.3	98.5	102.5	100.5	4833	3498	4165	81.4	73.2	77.3	51.2	49.7	50.4	357	322	62	247	10.8	15.9	18.0	14.9
Commander	S	61.5	58.0	59.8	71.0	76.5	73.8	3276	2521	2899	83.1	70.9	77.0	51.4	53.6	52.5	460	360	62	294	12.0	12.9	18.6	14.5
Eurostar	S	61.0	59.0	60.0	92.5	95.0	93.8	3322	2655	2988	84.0	74.0	78.9	46.9	49.1	48.0	433	366	85	295	11.1	12.8	18.8	14.2
Strongfield	S	59.0	58.5	58.8	92.0	86.0	89.0	5261	2200	3730	83.0	74.4	78.7	44.2	46.2	45.2	414	357	72	281	12.5	14.8	19.6	15.6
CDCVerona	S	60.0	59.0	59.5	91.5	96.5	94.0	4394	3120	3757	84.1	75.3	79.7	47.3	49.0	48.2	413	359	103	292	11.3	13.5	19.3	14.7
DT780	R	59.5	59.0	59.3	90.0	94.0	92.0	5288	4202	4745	82.3	74.5	78.4	42.7	48.1	45.4	403	353	63	273	11.7	13.9	18.4	14.7
Mean (pop.)		59.4	58.7	59.0	91.5	93.2	92.4	5025	3363	4194	82.6	74.1	78.4	46.4	46.7	46.6	404	343	81.6	276	12.0	14.5	18.6	15.0
Max (pop.)		61.5	61.0	61.3	103.5	105.5	101.3	6593	5307	5707	85.1	77.8	80.1	53.3	55.4	52.1	471	412	163.5	327	14.1	17.2	20.9	16.9
Min (pop.)		56.5	56.0	56.3	80.5	76.0	81.3	3520	1774	3223	80.2	70.3	76.2	38.9	40.7	41.0	318	260	61.0	215	10.3	12.6	15.5	13.2
LSD $_{0.05}$		1.80	1.40	1.40	7.40	12.6	7.80	1330	878	1615	1.00	1.30	6.90	3.70	3.60	3.20	52.0	41.0	25.0	176	1.60	2.00	0.900	3.60

Appendix 3: Least significant difference (LSD) and least square means (LSM) of yellow pigment (YP), wet gluten content (WETG), gluten index (GI), stretching time (ST) and relaxation (RELAX) for check cultivars and the CDC Verona/DT780 mapping population across three environments (Indian Head 2009, Kernen 2009 and Kernen 2010).

Line	S/R	YP				GI				WETG				ST				RELAX			
		IH09	KN09	KN10	COMBINED	IH09	KN09	KN10	COMBINED	IH09	KN09	KN10	COMBINED	IH09	KN09	KN10	COMBINED	IH09	KN09	KN10	COMBINED
D05M80001	S	5.4	6.6	7.7	6.6	19.2	21.5	24.4	21.7	27.7	36.3	46.7	36.9	6.0	6.0	6.0	6.0	260	251	225	245
D05M80002	S	6.5	7.9	8.4	7.6	78.7	50.2	33.6	54.2	18.6	33.5	42.4	31.5	19.0	9.0	6.0	11.3	214	246	245	235
D05M80003	S	5.6	6.0	7.9	6.5	89.4	78.2	65.3	77.6	22.7	27.5	41.1	30.4	28.0	26.0	19.5	24.5	207	208	206	207
D05M80004	S	6.4	7.9	8.3	7.5	31.4	65.3	33.2	43.3	27.1	30.5	46.7	34.8	6.0	43.0	6.0	18.3	258	184	250	231
D05M80005	S	5.7	6.7	8.3	6.9	54.9	50.7	46.7	50.7	23.0	29.5	43.6	32.0	9.5	25.5	10.0	15.0	250	200	241	230
D05M80006	S	5.8	6.7	7.7	6.7	56.2	69.6	42.2	56.0	21.4	27.0	42.5	30.3	13.5	15.0	7.5	12.0	225	231	234	230
D05M80007	R	6.0	6.7	8.3	7.0	35.9	29.8	26.9	30.9	22.1	31.4	41.1	31.5	7.5	7.5	6.0	7.0	261	258	233	250
D05M80008	S	5.2	6.3	8.1	6.5	46.0	10.3	27.2	27.8	29.2	37.9	42.4	36.5	7.0	6.0	6.0	6.3	268	287	265	273
D05M80010	S	6.3	7.4	8.7	7.4	45.1	49.5	33.6	42.7	28.3	35.6	48.1	37.3	6.0	8.0	6.0	6.7	264	252	263	259
D05M80012	S	5.9	6.9	9.1	7.3	86.5	55.7	45.4	62.5	19.5	31.7	44.5	31.9	27.0	11.0	9.0	15.7	212	238	237	229
D05M80014	S	6.3	6.9	8.7	7.3	19.6	23.6	23.5	22.3	27.5	33.7	47.1	36.1	6.0	11.5	6.0	7.8	258	235	225	239
D05M80015	R	6.9	7.7	9.2	7.9	21.0	25.6	24.8	23.8	22.9	33.0	39.0	31.6	7.0	9.5	6.0	7.5	240	223	216	226
D05M80016	S	6.6	7.6	9.7	8.0	51.0	53.9	38.5	47.8	20.7	26.4	43.9	30.3	9.5	8.5	6.0	8.0	237	260	253	250
D05M80017	R	5.8	6.9	7.6	6.8	52.7	40.2	36.1	43.0	21.8	36.7	41.5	33.3	13.5	7.0	6.0	8.8	229	243	247	239
D05M80018	R	7.2	8.3	8.8	8.1	68.8	46.9	31.9	49.2	21.4	33.4	43.6	32.7	10.5	7.5	6.0	8.0	242	242	243	242
D05M80019	S	5.5	6.0	7.2	6.2	43.3	20.2	36.2	33.2	24.1	33.7	37.5	31.7	7.5	6.0	6.0	6.5	263	273	262	266
D05M80021	S	5.5	6.6	7.8	6.6	10.5	31.8	27.6	23.3	25.6	36.9	45.5	36.0	6.0	7.0	6.0	6.3	277	224	211	237
D05M80022	S	6.9	7.9	8.8	7.9	69.0	58.6	47.2	58.3	23.4	32.8	46.7	34.3	22.0	10.5	6.5	13.0	220	247	254	240
D05M80023	R	7.0	8.0	9.7	8.2	47.9	42.1	27.5	39.2	19.2	28.5	38.3	28.6	7.5	6.0	6.0	6.5	242	263	244	249
D05M80024	R	8.2	9.4	9.5	9.0	35.0	58.5	12.8	35.4	24.9	27.4	37.3	29.9	6.5	11.0	6.0	7.8	247	248	222	239
D05M80025	S	6.4	7.2	7.9	7.2	79.2	65.4	60.3	68.3	23.2	29.0	37.7	29.9	33.5	20.0	24.5	26.0	205	240	199	215
D05M80026	R	8.2	8.9	9.6	8.9	72.9	75.8	39.1	62.6	22.8	30.1	44.8	32.6	18.5	24.5	6.0	16.3	221	204	239	221
D05M80027	S	6.6	8.0	9.2	7.9	77.6	82.7	59.3	73.2	25.4	22.4	41.2	29.6	20.5	35.0	20.0	25.2	233	202	212	216
D05M80028	S	6.5	7.2	8.4	7.4	88.4	78.6	62.1	76.3	20.2	26.2	36.9	27.7	32.0	28.5	34.5	31.7	214	201	184	200

D05M80030	S	6.3	7.1	8.3	7.2	62.8	58.9	49.0	56.9	27.3	31.9	49.8	36.3	12.5	15.0	7.5	11.7	229	227	249	235
D05M80032	S	5.6	6.6	7.2	6.4	47.0	53.1	23.7	41.3	25.1	30.8	38.4	31.4	7.0	9.5	6.0	7.5	275	261	256	264
D05M80033	S	6.3	7.4	8.1	7.3	61.0	62.9	50.3	58.1	25.4	30.4	42.7	32.8	11.0	16.0	10.0	12.3	258	227	242	242
D05M80034	S	6.4	6.8	9.3	7.5	83.7	56.0	59.1	66.3	22.7	30.8	40.2	31.2	23.0	12.5	20.5	18.7	225	246	209	227
D05M80039	R	6.3	7.0	8.4	7.2	23.5	43.7	33.8	33.7	24.0	31.2	42.8	32.7	7.0	17.0	6.0	10.0	270	219	247	245
D05M80041	R	6.0	6.7	8.5	7.1	50.4	40.9	27.6	39.6	20.3	30.4	37.2	29.3	13.0	6.0	6.0	8.3	231	264	232	242
D05M80042	R	6.3	7.0	8.5	7.3	66.3	49.2	43.0	52.8	18.3	30.3	35.1	27.9	16.5	10.5	10.5	12.5	236	238	231	235
D05M80043	R	6.4	8.3	8.9	7.9	38.9	39.5	24.3	34.2	26.9	34.5	42.8	34.7	7.5	6.0	6.0	6.5	257	262	206	242
D05M80045	S	7.1	8.1	9.1	8.1	36.2	57.0	46.0	46.4	27.1	27.6	42.2	32.3	7.0	13.5	9.0	9.8	253	243	244	247
D05M80048	S	6.9	7.1	8.6	7.5	29.4	16.4	23.9	23.2	20.3	34.7	44.7	33.2	7.0	6.0	6.0	6.3	254	276	252	261
D05M80049	S	6.0	6.6	8.8	7.1	43.7	32.9	31.8	36.1	26.1	32.8	48.1	35.6	9.5	6.0	6.0	7.2	249	266	264	260
D05M80050	S	7.2	8.3	9.0	8.2	44.2	28.5	26.4	33.1	24.9	30.6	47.0	34.1	8.0	7.0	6.0	7.0	254	271	244	256
D05M80053	S	6.2	6.8	8.2	7.1	85.9	55.3	44.8	62.0	17.1	26.4	37.3	26.9	56.0	12.0	8.0	25.3	177	248	242	222
D05M80054	S	6.1	7.0	8.5	7.2	41.6	43.2	34.1	39.6	28.3	36.4	46.1	36.9	9.0	8.0	6.0	7.7	247	247	249	247
D05M80055	R	7.0	7.9	8.9	8.0	41.0	34.3	25.6	33.6	29.2	37.3	40.3	35.6	6.0	6.0	6.0	6.0	261	248	239	249
D05M80056	R	6.7	8.0	8.6	7.8	34.5	30.0	22.6	29.0	23.3	36.9	42.2	34.1	6.0	6.0	6.0	6.0	273	272	229	258
D05M80057	S	6.7	8.0	9.0	7.9	61.9	64.2	45.5	57.2	22.6	29.8	47.7	33.3	14.0	14.0	10.0	12.7	238	230	240	236
D05M80058	S	6.4	7.4	8.6	7.4	65.2	42.3	43.1	50.2	18.8	30.5	43.3	30.9	7.5	6.0	6.0	6.5	257	253	260	256
D05M80059	R	6.2	7.0	8.3	7.2	35.5	38.4	40.9	38.3	26.8	41.4	44.5	37.6	6.5	6.0	6.0	6.2	250	258	263	257
D05M80060	S	6.3	7.4	9.7	7.8	50.2	41.2	48.4	46.6	27.1	33.9	43.4	34.8	8.5	7.0	11.5	9.0	254	258	231	248
D05M80063	S	5.9	7.1	7.5	6.8	46.1	50.5	41.2	46.0	22.4	29.6	49.6	33.9	12.0	7.5	6.0	8.5	238	260	262	253
D05M80064	S	5.8	7.0	8.1	7.0	4.5	7.4	21.7	11.2	28.7	35.6	46.8	37.0	6.0	6.0	6.0	6.0	263	260	226	249
D05M80067	S	7.1	7.9	9.3	8.1	60.3	45.5	56.9	54.2	26.7	34.3	37.2	32.7	14.0	10.0	26.5	16.8	235	237	208	226
D05M80068	R	7.3	9.0	9.3	8.6	34.3	39.1	20.8	31.4	26.2	40.8	41.9	36.3	6.0	6.0	6.0	6.0	259	254	228	247
D05M80069	S	6.3	7.5	7.9	7.2	74.0	53.3	47.1	58.1	22.1	27.5	45.5	31.7	14.5	25.5	9.5	16.5	243	218	238	233
D05M80070	S	6.3	7.0	8.8	7.4	80.2	66.7	51.3	66.1	24.7	30.7	42.3	32.6	22.0	17.5	14.5	18.0	219	223	221	221
D05M80071	R	7.1	8.4	9.1	8.2	42.0	53.8	22.5	39.4	25.6	33.0	43.0	33.8	9.5	56.5	6.0	24.0	249	170	243	220
D05M80074	S	5.3	5.6	8.7	6.5	49.7	41.2	36.9	42.6	27.0	32.3	42.4	33.9	7.5	6.0	6.0	6.5	273	270	254	265
D05M80076	S	7.1	8.1	9.0	8.1	69.4	61.7	47.4	59.5	21.8	28.9	45.4	32.0	23.0	67.5	9.5	33.3	210	157	237	201
D05M80077	R	6.7	7.7	8.5	7.6	76.9	64.4	39.5	60.3	18.9	29.0	39.4	29.1	18.0	12.0	6.0	12.0	222	240	239	234

D05M80078	R	7.3	8.3	8.9	8.2	57.0	59.1	43.3	53.1	25.0	35.1	40.1	33.4	11.0	15.5	8.5	11.7	246	223	240	236
D05M80079	S	5.8	6.6	8.3	6.9	64.1	48.9	39.2	50.7	23.0	32.6	39.6	31.7	16.5	9.0	6.0	10.5	227	250	243	240
D05M80080	R	6.2	7.5	8.7	7.4	25.1	34.2	17.4	25.6	24.0	34.0	41.0	33.0	6.0	8.0	6.0	6.7	254	248	246	249
D05M80081	S	5.5	6.0	7.9	6.5	32.4	39.2	28.8	33.5	24.3	33.5	42.1	33.3	7.0	6.0	6.0	6.3	262	262	242	255
D05M80082	R	6.5	7.3	8.8	7.5	71.6	57.3	18.8	49.2	22.2	29.0	39.8	30.3	20.0	14.0	6.0	13.3	217	230	243	230
D05M80083	R	7.1	7.8	9.1	8.0	68.6	39.9	35.1	47.8	18.7	32.4	40.1	30.4	15.0	7.5	6.0	9.5	215	252	246	237
D05M80084	S	5.6	6.7	7.4	6.6	30.2	47.5	30.0	35.9	26.1	34.0	46.9	35.7	6.0	8.5	6.0	6.8	247	244	254	248
D05M80086	S	5.9	6.6	7.3	6.6	69.8	75.6	61.6	69.0	23.3	26.1	36.3	28.6	29.0	71.5	46.0	48.8	200	168	169	179
D05M80087	S	5.2	6.2	8.1	6.5	76.6	39.7	49.1	55.1	20.0	31.5	40.6	30.7	16.0	6.5	10.5	11.0	238	270	236	248
D05M80088	S	6.7	7.7	9.7	8.0	59.2	52.3	47.2	52.9	24.3	31.9	43.4	33.2	19.0	13.0	12.0	14.7	219	235	223	226
D05M80089	S	5.8	6.7	8.0	6.8	69.5	20.5	39.1	43.0	19.5	31.5	39.6	30.2	11.5	6.0	6.5	8.0	244	265	266	258
D05M80090	S	6.1	7.3	9.4	7.6	36.1	36.4	28.6	33.7	23.2	31.0	39.8	31.3	7.0	8.0	6.0	7.0	259	250	251	253
D05M80091	R	7.2	7.9	9.3	8.1	33.4	33.0	28.5	31.6	24.5	31.2	41.5	32.3	6.0	6.0	6.0	6.0	276	286	272	278
D05M80092	R	5.9	6.8	8.7	7.1	44.5	14.2	30.0	29.5	20.7	36.2	40.6	32.5	7.0	6.0	6.0	6.3	261	257	256	258
D05M80095	S	6.0	6.9	8.2	7.0	48.6	36.3	30.0	38.3	23.2	30.2	43.0	32.1	11.5	9.5	6.0	9.0	231	240	244	238
D05M80096	S	5.7	7.0	8.8	7.2	79.3	68.9	55.6	67.9	23.1	25.2	41.5	29.9	26.5	17.0	14.5	19.3	203	224	221	216
D05M80097	S	6.2	7.1	9.0	7.4	45.0	59.8	29.4	44.7	30.2	33.6	43.2	35.6	9.5	15.0	6.0	10.2	251	236	214	234
D05M80098	S	6.5	7.9	8.6	7.7	58.4	77.6	41.1	59.0	21.5	26.6	49.0	32.3	11.5	64.0	6.0	27.2	237	165	257	220
D05M80100	R	7.3	8.0	9.4	8.2	56.0	52.0	19.8	42.6	22.6	31.3	41.5	31.8	11.5	10.0	6.0	9.2	234	246	225	235
D05M80101	S	6.9	8.0	8.5	7.8	40.6	64.6	47.9	51.0	25.4	33.4	43.4	34.1	7.0	11.5	7.5	8.7	252	232	250	244
D05M80102	R	7.0	7.4	9.1	7.8	65.4	57.2	30.9	51.2	22.7	33.6	41.0	32.4	13.5	15.5	6.0	11.7	231	219	219	223
D05M80103	S	7.1	8.1	8.4	7.9	70.9	77.2	51.9	66.7	22.2	26.9	46.3	31.8	20.0	21.0	15.0	18.7	217	225	225	222
D05M80104	S	6.3	6.4	9.5	7.4	57.8	45.9	44.0	49.2	25.0	33.6	42.3	33.6	8.5	8.5	12.5	9.8	261	255	221	245
D05M80105	S	6.8	8.0	9.5	8.1	60.3	54.9	61.3	58.8	25.4	30.8	39.5	31.9	12.5	10.0	18.0	13.5	239	252	215	235
D05M80107	S	5.9	6.7	8.6	7.1	71.6	65.7	66.3	67.9	24.2	29.8	37.8	30.6	28.5	27.0	38.5	31.3	204	197	176	192
D05M80108	S	6.4	7.3	8.8	7.5	80.4	55.4	38.3	58.0	22.8	30.6	48.5	33.9	16.5	8.5	6.0	10.3	229	259	263	250
D05M80109	S	6.5	7.2	9.1	7.6	45.3	64.3	39.8	49.8	20.1	29.5	44.3	31.3	7.5	11.0	6.0	8.2	254	236	259	250
D05M80110	S	7.1	8.0	9.0	8.0	87.5	75.6	48.0	70.3	117.8	28.8	48.9	65.1	18.5	14.5	9.5	14.2	221	250	240	237
D05M80112	S	6.5	7.5	8.4	7.5	53.2	43.0	39.9	45.3	23.0	33.6	49.2	35.3	8.5	9.5	6.0	8.0	249	269	266	261
D05M80116	S	5.6	7.0	7.6	6.7	64.1	46.8	42.9	51.3	24.7	33.6	41.8	33.3	18.5	6.5	7.0	10.7	228	263	248	246

D05M80117	S	6.3	7.3	9.3	7.6	76.5	64.3	60.0	66.9	21.5	36.0	43.2	33.6	29.5	27.5	17.5	24.8	205	198	207	203
D05M80119	R	7.3	8.1	8.8	8.1	59.1	31.3	35.4	41.9	21.9	31.9	38.9	30.9	9.0	6.0	6.0	7.0	251	269	254	258
D05M80120	S	6.6	6.7	9.1	7.5	63.5	61.4	53.0	59.3	23.0	35.3	41.5	33.2	14.0	16.0	20.5	16.8	238	215	203	219
D05M80121	S	6.9	7.8	9.4	8.0	45.2	37.9	20.1	34.4	24.9	29.8	42.2	32.3	7.5	7.5	6.0	7.0	266	247	238	250
D05M80122	R	6.3	6.8	8.4	7.2	7.8	14.7	29.2	17.2	25.0	38.5	42.2	35.2	6.0	6.0	6.0	6.0	252	248	221	240
D05M80123	S	6.2	7.1	7.9	7.0	49.4	47.2	51.0	49.2	30.6	34.3	46.5	37.1	8.0	9.0	8.0	8.3	274	268	252	265
D05M80124	S	6.5	7.4	9.0	7.7	31.1	39.2	36.8	35.7	28.1	34.4	44.2	35.5	6.5	6.5	6.0	6.3	264	268	243	258
D05M80125	S	6.8	7.7	9.0	7.8	27.5	28.8	28.9	28.4	27.2	33.8	46.4	35.8	6.0	6.0	6.0	6.0	282	253	244	259
D05M80126	R	7.2	8.3	9.2	8.2	28.8	14.2	31.1	24.7	28.9	38.5	45.3	37.6	7.5	6.0	6.0	6.5	261	251	242	251
D05M80127	S	5.7	6.8	8.5	7.0	53.7	69.8	41.4	54.9	24.2	26.5	45.6	32.1	11.0	14.0	6.5	10.5	242	230	243	238
D05M80129	S	5.4	6.5	8.2	6.7	75.9	40.0	40.4	52.1	20.9	30.0	41.1	30.6	27.5	6.5	7.0	13.7	207	272	262	247
D05M80130	S	6.4	7.7	9.1	7.7	47.4	26.9	15.6	29.9	20.8	29.4	43.2	31.1	8.0	6.0	6.0	6.7	249	278	217	248
D05M80131	S	6.9	8.0	9.4	8.1	65.7	49.8	47.8	54.4	22.7	35.0	52.6	36.7	19.5	8.5	6.5	11.5	219	256	251	242
D05M80134	R	6.2	6.9	9.1	7.4	28.9	16.5	28.2	24.5	27.6	38.8	42.4	36.3	7.5	6.0	6.0	6.5	244	259	220	241
D05M80135	S	6.2	7.2	8.3	7.2	73.5	57.7	58.9	63.4	21.0	33.2	44.4	32.9	18.0	13.5	15.0	15.5	223	226	220	223
D05M80136	S	6.5	7.1	9.4	7.7	43.4	54.6	51.8	49.9	28.5	34.9	42.0	35.1	7.5	12.5	11.5	10.5	276	233	226	245
D05M80137	S	6.1	7.1	9.0	7.4	35.5	64.2	35.9	45.2	23.0	31.9	41.4	32.1	9.0	11.0	6.5	8.8	246	229	243	239
D05M80140	S	6.3	6.9	8.1	7.1	71.2	73.3	48.2	64.2	26.5	29.2	46.5	34.0	24.0	26.5	7.0	19.2	218	201	251	223
D05M80141	S	6.6	7.4	9.0	7.7	81.6	68.8	65.2	71.8	19.7	33.5	39.8	31.0	38.0	27.5	26.5	30.7	194	200	194	196
D05M80142	S	7.2	8.0	9.5	8.2	8.9	28.8	29.6	22.4	24.9	32.1	45.1	34.0	6.0	6.0	6.0	6.0	253	267	201	240
D05M80143	R	6.0	7.0	8.4	7.1	57.2	53.1	39.1	49.8	24.9	31.5	42.8	33.0	10.5	14.0	6.0	10.2	254	234	246	244
D05M80144	R	6.1	7.1	7.9	7.0	33.6	42.4	19.5	31.8	26.0	29.5	39.4	31.6	6.0	8.5	6.0	6.8	269	248	238	252
D05M80146	S	6.5	7.1	9.3	7.6	43.2	36.6	33.7	37.8	28.0	42.5	48.6	39.7	6.0	7.0	6.0	6.3	265	250	244	253
D05M80147	S	6.7	7.7	8.4	7.6	12.3	14.6	28.5	18.4	26.4	37.2	46.2	36.6	6.0	6.0	6.0	6.0	285	278	260	274
D05M80148	S	6.7	8.0	9.4	8.0	73.2	49.8	40.1	54.4	21.7	30.1	39.2	30.3	15.0	8.0	7.0	10.0	234	259	239	244
D05M80149	S	6.0	7.0	8.4	7.1	68.2	62.8	57.1	62.7	24.1	31.7	41.2	32.3	27.0	25.0	15.0	22.3	212	212	229	218
D05M80151	S	6.9	8.0	9.5	8.1	83.6	66.7	52.3	67.5	22.8	30.3	43.2	32.1	25.5	29.0	15.5	23.3	215	199	219	211
D05M80154	S	7.8	8.7	9.4	8.6	53.8	43.0	44.1	47.0	25.7	40.5	43.2	36.4	12.0	9.0	7.5	9.5	248	235	244	242
D05M80155	S	6.8	8.1	9.2	8.0	60.2	58.1	49.5	55.9	27.9	31.1	49.8	36.2	13.5	9.5	8.5	10.5	240	255	251	249
D05M80156	R	6.9	8.1	9.1	8.0	40.9	32.0	19.7	30.9	21.6	36.4	42.2	33.4	9.0	6.0	6.0	7.0	243	258	234	245

D05M80157	S	5.7	6.8	9.5	7.3	52.3	49.8	31.9	44.6	24.3	32.1	43.3	33.2	9.5	53.0	6.0	22.8	261	177	245	228
D05M80158	S	7.0	7.9	8.9	8.0	49.7	41.4	36.5	42.5	26.2	31.8	45.6	34.5	14.5	6.5	6.0	9.0	235	252	239	242
D05M80159	S	6.8	7.6	9.0	7.8	46.6	55.9	48.5	50.3	22.3	27.7	43.3	31.1	9.5	9.5	12.0	10.3	235	241	225	233
D05M80160	S	7.0	7.7	9.3	8.0	46.1	32.7	33.0	37.3	24.5	35.6	41.5	33.8	8.0	6.0	7.0	7.0	275	263	253	263
Goodeve	R	2.4	3.2	3.5	3.0	61.2	52.1	49.1	54.1	32.3	36.7	53.7	40.9	15.5	9.0	6.0	10.2	221	257	266	248
Waskada	S	0.6	1.0	2.4	1.3	62.5	55.2	44.6	54.1	33.4	41.0	51.9	42.1	11.0	22.5	6.0	13.2	258	219	263	246
ACAvonlea	S	5.8	6.7	8.2	6.9	25.8	40.0	32.0	32.6	27.7	36.1	45.7	36.5	6.0	7.0	6.0	6.3	288	234	255	259
ACMorse	S	5.2	6.5	7.7	6.5	46.1	37.9	48.0	44.0	24.5	33.8	43.4	33.9	7.5	6.0	9.0	7.5	271	276	249	265
ACNavigator	S	6.2	7.3	9.2	7.6	80.0	74.6	44.2	66.3	22.5	28.4	41.7	30.9	28.0	15.0	9.0	17.3	202	229	230	220
Brigade	S	6.4	7.7	8.5	7.5	96.1	69.6	64.3	76.7	17.6	34.2	39.6	30.4	80.5	21.5	16.5	39.5	178	210	223	203
Commander	S	6.5	8.0	8.9	7.8	96.9	85.9	53.9	78.9	22.4	27.8	42.5	30.9	76.5	46.5	18.0	47.0	175	184	215	191
Eurostar	S	6.1	6.7	8.1	7.0	89.6	77.8	53.0	73.4	19.7	24.0	44.4	29.3	35.5	23.0	15.5	24.7	197	221	222	213
Strongfield	S	6.6	7.4	8.7	7.6	49.6	46.6	46.5	47.6	26.6	33.2	45.7	35.1	9.5	8.0	9.5	9.0	252	246	240	246
CDCVerona	S	6.3	7.6	8.7	7.5	74.6	58.0	45.8	59.5	20.3	31.0	48.3	33.2	18.0	14.5	7.0	13.2	225	236	256	239
DT780	R	6.7	7.7	8.5	7.6	48.3	42.2	39.3	43.3	24.6	31.5	43.1	33.0	7.5	6.0	6.0	6.5	261	259	245	255
Mean (pop.)		6.5	7.3	8.7	7.5	52.8	47.7	38.6	46.4	24.7	32.2	42.9	33.3	13.2	13.8	9.3	12.1	242	240	236	239
Max (pop.)		8.2	9.4	9.7	9.0	89.4	82.7	66.3	77.6	117.8	42.5	52.6	65.1	56.0	71.5	46.0	48.8	285	287	272	278
Min (pop.)		5.2	5.6	7.2	6.2	4.5	7.4	12.8	11.2	17.1	22.4	35.1	26.9	6.0	6.0	6.0	6.0	177	157	169	179
LSD $_{0.05}$		0.4	0.550	0.592	1.20	17.5	23.4	8.15	14.8	24.1	7.36	2.90	12.7	18.5	30.0	6.51	12.8	32.0	55.0	21.0	25.0